BODY CONDITION AND REPRODUCTIVE STRATEGIES OF FEMALE LESSER SCAUP IN THE BOREAL FOREST OF ALASKA

By

Kristin A. DeGroot

RECOMMENDED:

Joel Schmutz
Advisory Committee Member

Mark Lindberg
Advisory Committee Co-chair

Perry Barboza
Advisory Committee Co-chair

Mark Lindberg
Chair, Department of Biology and Wildlife

APPROVED:

Paul Layer
Dean, College of Natural Science and Mathematics

Lawrence Duffy
Interim Dean of the Graduate School

Apr 5, 2011
Date

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A

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Kristin A. DeGroot, B.S.

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ABSTRACT

In many waterfowl species, body condition of breeding females can contribute to reproductive success by influencing factors such as egg size, clutch size and ability to incubate eggs. In turn, changes in female condition at the population level could affect population growth rates. Large-scale declines in populations of Lesser Scaup (*Aythya affinis*) raised concerns that poor female body condition was contributing to declines by reducing reproductive output. However, little was known about changes in body condition over time and about the contribution that stored body reserves make to egg production, especially in boreal forest regions where most scaup breed. My objectives were: 1) examine temporal changes in body condition of pre-breeding female lesser scaup on the Yukon Flats National Wildlife Refuge, Alaska and the relationship between body condition and breeding status; 2) examine the role of body reserves (protein and lipid) in egg production using stable isotope techniques. I found no evidence for a decline in female body condition as compared to historic measures. However, females that had entered rapid follicle growth (the early stages of egg production) were significantly fatter than birds that were not currently producing eggs. In addition, I found that female lesser scaup use both body reserves and dietary nutrients for production of egg yolk.

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INTRODUCTION

Changes in avian populations over time can be described by the demographic parameters of birth rate, death rate, immigration and emigration. At large spatial scales (i.e., the population level), immigration and emigration are minimal. Therefore, population size is primarily driven by survival (deaths) and recruitment (births). These parameters, in turn, are governed by extrinsic factors such as food supply, predation and disease (Newton 1998). Therefore, when one or several extrinsic factors affect survival and recruitment, populations grow or decline. For example, increases in the continental population of Lesser Snow Geese (*Chen caerulescens caerulescens*) have been attributed to greater food availability from agricultural practices (Alisauskas and Ankney 1992b). Additional food increases winter survival. Therefore, more birds breed in the spring and more young are recruited into the population. Population-level declines in birds can be attributed to intra- and inter-specific competition, habitat loss, predation, diseases and parasites, weather events, and human-caused mortality, among others (Newton 1998).

Extrinsic factors limit reproductive output in birds by influencing parameters such as breeding propensity, nest success, frequency of renesting, clutch size and chick survival. Wildlife biologists quantify reproductive output in bird populations and manipulate extrinsic factors such as habitat quality and quantity, predation, inter- or intra-specific competition to achieve management goals. For example, managers successfully increased duck recruitment in the prairie pothole region of the U.S. through the Conservation Reserve Program, a program that offers landowners financial incentive for converting cropland to perennial grassland (Reynolds et al. 2001). In addition, managers in Michigan successfully stabilized the decreasing and endangered Kirtland's Warbler (*Dendroica kirtlandii*) population by controlling the Brown-headed Cowbird (*Molothrus ater*; Kelly and DeCapita 1982), an inter-specific nest competitor. Other extrinsic factors that influence reproductive output but are largely outside the bounds of management include weather events and nest initiation date.

Health and condition of female birds can also affect reproductive output. Females carry the burden of producing energy-dense eggs and, in many species, may be solely responsible for incubating eggs and feeding, protecting and caring for young. In addition, incubating females may eat very little while incubating and, in some species, may incubate almost continuously (Common Eider [*Somateria mollissima*]; Goudie et al. 2000). Therefore, poor female health or nutrition can have consequences for reproduction and may affect populations, if poor health influences reproductive output on a large scale.

Body condition can be defined as the relative mass of the whole bird and/or mass of respective components of fat (lipid) and protein. In many species, body condition can directly influence reproductive output. Birds in better condition in the spring are more likely to breed, breed more often, have larger clutches, lay larger eggs and are more successful at hatching eggs (Ankney et al. 1991; Alisauskas and Ankney 1992a). Therefore, poor female condition at the population level (resulting from habitat loss, poor food quality or quantity, increased energetic demands due to predator pressure, etc.) could have detrimental effects on subsequent reproductive output and, therefore, recruitment.

Body condition is especially important for those species that allocate stored reserves directly to eggs or require stored energy for incubation. Typically, species are classified along a 'capital/income' continuum, with capital breeders primarily relying on stored nutrients (such as those acquired on wintering or staging areas) for egg production and income breeders using nutrients acquired on breeding areas (Meijer and Drent 1999). Reproduction in capital breeders or mixed capital/income breeders may be ultimately limited by reserves acquired on wintering or staging areas and, therefore, body condition of birds upon arrival to breeding grounds may determine subsequent reproductive performance.

As a taxa, waterfowl have been well studied with respect to nutrient investment in reproduction. Waterfowl are relatively large-bodied birds that invest proportionately large amounts of nutrients into energy-dense eggs (Alisauskas and Ankney 1992a). For

this reason, body condition of pre-breeding females has been thought to influence subsequent reproductive output (Alisauskas and Ankney 1992a; Webster et al. 2002). In addition, waterfowl species employ a diverse range of energetic tactics to meet the demands of egg production and incubation. Although a few species rely almost entirely on body capital (Emperor Goose [*Chen canagica*], Schmutz et al. 2006) or dietary income (Northern Shoveler [*Anas clypeata*], MacCluskie and Sedinger 2000; Redhead [*Aythya americana*], Hobson et al. 2004; King Eider [*Somateria spectabilis*], Lawson 2006 and Oppel 2008; Greater Scaup [*Aythya marila*], Gorman et al. 2008) to produce eggs, many species rely on a mix of capital and income for reproduction (Greater Snow Goose [*Chen caerulescens atlantica*], Gauthier et al. 2003; Barrow's Goldeneye [*Bucephala islandica*], Hobson et al. 2005; Long-tailed Duck [*Clangula hyemalis*], Lawson 2006; Black Brant [*Branta bernicla*] Schmutz et al. 2006).

Understanding the relative significance of body stores for reproduction in waterfowl is important for managing populations because it can direct management decisions and resources by helping to identify geographic areas vital for nutrient acquisition. Identifying these factors may be especially important for species showing long-term population declines, such as sea ducks (Scoters, *Melanitta* spp. and Eiders, *Somateria* and *Polysticta* spp.).

The combined populations of Lesser (*Aythya affinis*) and Greater (*A. marila*) Scaup have been declining across North America since the early 1980's (Afton and Anderson 2001; Zimpfer et al. 2009). Annual US Fish and Wildlife Service surveys indicate that scaup populations dropped by approximately 150,000 birds per year between 1978-1997, and are now 18% below the long term average (Zimpfer et al. 2009). These declines are of particular concern for lesser scaup that constitute up to 89% of the continental population (Belrose 1980).

One hypothesis for these declines suggests that poor body condition of breeding females may explain reduced reproductive output (the spring condition hypothesis [SCH]; Austin et al. 2000; Afton and Anderson 2001; Anteau and Afton 2004). Although support for the SCH has been documented at northern sites along the Mississippi Flyway,

few studies have investigated condition of females in the boreal forest (DeVink et al. 2008), where most (68%) lesser scaup breed (Austin et al. 1998).

This study examined body condition, reproductive status and nutrient investment strategies of Lesser Scaup (hereafter, scaup) on the Yukon Flats National Wildlife Refuge (YFNWR). The YFNWR is located in interior Alaska and encompasses approximately 36,500 km^2 of boreal forest bisected by the Yukon River. The landscape is classified as 48% wetland, much of it closed-basin (USFWS 1987) and supports large numbers of breeding birds including waterfowl, shorebirds, passerines, and raptors. The YFNWR is an important breeding area for the continental population of lesser scaup, with approximately 166,000 scaup (greater and lesser combined) counted during the annual U.S. Fish and Wildlife Service breeding bird survey in 2007 (N. Guldager, pers. comm.). Scaup are the most abundant duck on the YFNWR and have been well studied: intensive banding efforts began in the 1950's (King 1963) and other work has examined nutrient dynamics (Esler et al. 2001), nest success, duckling survival (Corcoran et al. 2007) and, most recently, breeding probability and survival of females (Martin 2007; Martin et al. 2009).

The main objectives of this study were twofold. First, I examined temporal shifts in body condition of pre-breeding female lesser scaup collected on the YFNWR in 2007 and 2008 with measures taken at the same site in 1991. I also investigated intraspecific variation in condition of females relative to reproductive status (Chapter 1). Second, I used naturally occurring isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) to examine the importance of endogenous (body capital) and exogenous (dietary income) nutrients allocated to Lesser Scaup egg yolk. I also investigated intraspecific and inter-female variation in nutrient allocation strategies (Chapter 2).

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CHAPTER 1. BODY CONDITION OF FEMALE LESSER SCAUP IN THE BOREAL FOREST OF ALASKA: TEMPORAL VARIATION AND NUTRIENT RESERVE DYNAMICS[1]

ABSTRACT

Continental populations of Lesser Scaup (*Aythya affinis*; hereafter, scaup), a medium-sized diving duck, have been declining since the early 1980's and declines are particularly pronounced in the boreal forest, where most scaup breed. One well-established hypothesis suggests that poor body condition of breeding females may explain reduced reproductive output. Therefore, it is important to understand relationships between body condition and reproductive parameters and also track changes in body condition over time. We examined temporal patterns in body condition of paired, pre-breeding female lesser scaup collected on the Yukon Flats National Wildlife Refuge, Alaska, USA in 2007 and 2008 (n = 36) with measures taken at the same site in 1991 (n = 33). We found no support for a decline in mass of any measured body components, including total body mass, protein, lipid and mineral ash. In addition, we investigated intra-specific variation in body condition of females relative to reproductive status. Females that had entered rapid follicle growth (RFG) were 80g heavier and carried 18g more lipid than birds that had not entered RFG; we found no effect of RFG status on protein or ash mass. Our results suggest that body condition of scaup in Alaska varies widely among years and has not changed significantly since 1991. However, large differences in lipid stores between RFG and non-RFG females may be indicative of nutrient limitations for initiation of breeding and could potentially influence breeding propensity at the population level.

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INTRODUCTION

Migratory birds use a variety of energetic strategies to meet the proximate demands of egg production (Meijer and Drent 1999). Typically, species have been classified along a continuum between capital and income that describes the relative importance of stored nutrients (body capital) and food (dietary income) for meeting the demands of egg production and incubation. Large-bodied species that breed at high latitudes, such as arctic-nesting geese, have been thought of as model capital breeders because they are capable of carrying large reserves and may arrive on breeding grounds prior to adequate food availability. In contrast, income breeders primarily meet the nutritional demands of reproduction with nutrients acquired on breeding grounds. Nutrient reserve dynamics and reproduction have been particularly well studied in waterfowl, with species employing a range of strategies along the capital/income continuum (Meijer and Drent 1999). For waterfowl that use stored fat and protein for egg formation and/or incubation, body condition of females in the spring may influence important reproductive parameters such as breeding propensity, egg size, clutch size, incubation constancy or nest success (Ankney et al. 1991; Alisauskas and Ankney 1992). Furthermore, poor body condition has been associated with low breeding propensity and reduced reproductive output in waterfowl (Alisauskas and Ankney 1992). Therefore, it is important to describe variation in reproductive strategies and how this variation may influence reproductive output. Understanding nutrient reserve dynamics may be particularly important for species experiencing population declines if nutrition ultimately influences population growth rates.

The combined populations of lesser (*Aythya affinis*) and greater (*A. marila*) scaup have been declining across North America since the early 1980s (Afton and Anderson 2001; Zimpfer et al. 2009). Annual surveys conducted by the US Fish and Wildlife Service show that scaup populations dropped by approximately 150,000 birds per year between 1978-1997, and are now 18% below the long term average (Zimpfer et al. 2009). These declines are of particular concern for lesser scaup (hereafter, scaup) that constitute up to 89% of the continental population (Belrose 1980). One hypothesis for these

declines suggests that poor body condition of breeding females may explain reduced reproductive output (the spring condition hypothesis [SCH]; Austin et al. 2000; Afton and Anderson 2001; Anteau and Afton 2004). Although support for the SCH has been documented at northern staging areas along the Mississippi Flyway, few studies have investigated condition of females in the boreal forest (DeVink et al. 2008), where most (68%) scaup breed (Austin et al. 1998), and where changes in body condition would be most relevant to that year's production. Therefore, we examined changes in body condition of females in the spring on the Yukon Flats National Wildlife Refuge, Alaska over a 16-17 year time span. In addition, we examined the relationship between female body condition and reproductive status, which was defined as rapid follicle growth (RFG), or the stage at which egg follicles begin to sequester yolk (Johnson 2000) .

Our objectives were to 1) examine temporal patterns in body condition of paired female lesser scaup collected in the spring on the Yukon Flats National Wildlife Refuge, Alaska in 2007 and 2008 with measures taken at the same site in 1991 and 2) investigate intra-specific variation in condition of females relative to reproductive status. We predicted that females collected in recent years would not show evidence for reduced condition compared to birds collected 16-17 years earlier because other work on scaup in the boreal forest of Canada found no decline in body condition (DeVink et al. 2008). Also, we expected that females in RFG would be heavier and carry more fat than females that had not commenced egg production.

Study Area

Our study took place on the Yukon Flats National Wildlife Refuge (Fig 1). The YFNWR encompasses approximately 36,500 km^2 of boreal forest bisected by the Yukon River. The landscape is classified as 48% wetland, much of it closed-basin (USFWS 1987). Lakes and ponds are surrounded by emergent vegetation (including bulrush [*Typha latifolia*]) and open meadows; upland habitats include stands of white spruce (*Picea glauca*), quaking aspen (*Populus tremuloides*), and paper birch (*Betula papyrifera*). These extensive wetlands support large numbers of breeding waterfowl. The YFNWR is home to the second largest concentration of breeding ducks in Alaska,

with over 100,000 pairs counted annually (Conant and Groves 2005). Scaup (lesser and greater combined) are the most abundant duck on the YFNWR and this area has been regarded as an important breeding area for the continental population of Lesser Scaup (USFWS 1986).

Our study area includes three sites. The Long Lake study site (66° 20' N, 147°58' W) was used most recently by Martin et al. (2009) to study breeding propensity and survival of female scaup. The Canvasback Lake site (66° 24' N, 146°23' W) is approximately 80km east of Long Lake. Historical use of this site includes studies of nest success (Grand 1995) and was also was also the location of scaup banding efforts in the 1960's (King 1963). The Yukon River site (66° 11' N, 147°41' W) spans approximately 30km of wetlands adjacent to the Yukon River south of Long Lake.

METHODS

Bird Collection

We collected paired female scaup from 29 to 31 May, 2007 and from 27 to 30 May, 2008, which is within the period between arrival and nest initiation on the YFNWR (hereafter, we refer to this as the pre-breeding period). Birds were killed with shotgun (12 gauge) or rifle (.222 or .223) at 14 distinct wetlands in 2007 (n =18) and 10 wetlands in 2008 (n = 29) without the aid of decoys (Weatherhead and Greenwood 1981; Fast et al. 2008). We collected birds at both the Yukon River and Canvasback Lake sites in 2008 and on the Yukon River site in 2007. Maximum distance between collection sites was 83 km. As soon as the carcass was retrieved, birds were weighed to the nearest 10g using a Pesola spring scale (Baar, Switzerland), then approximately 1ml of blood was collected directly from the ventricle of the heart with a 21 gauge needle and 2mL syringe (BD Vacutainer, Franklin Lakes, NJ). In 2008, portions of blood samples were centrifuged at 3,000 x *g* to separate plasma and cells. The reproductive tract was dissected and stored in 10% formalin. Carcasses were wrapped in two layers of plastic and two layers of freezer bags to retain moisture, then carcasses and blood were kept cool until samples could be stored frozen, usually within 12 – 48h.

We compared our data to those collected in 1991 at Long Lake (D. Esler, unpublished data). The span of data in 1991 were longer and generally later than those in 2007-08 so we used only those data collected during 8 May - 8 June, 1991 because these dates correspond to the typical pre-breeding period for scaup.

Bird Capture

At the Long Lake study site, we used female scaup and American Wigeon (*Anas americana*) as decoys to capture female lesser scaup (Anderson et al. 1980; Sharp and Lokemoen 1987). Decoy hens were held in indoor pens at the University of Alaska Fairbanks over winter and transported to Long Lake in May where they were maintained in a 4m x 4m pen with Duck and Goose Maintenance ration *ad libitum* (Alaska Mill and Feed Company, Anchorage, AK), which was supplemented with meal worms (Sunshine Mealworms, Silverton, Oregon). We used swim-in traps (1.5m x 1.5m) with decoys to capture birds from 21 May through 8 June, 2007 and from 16 May to 4 June, 2008. Ten to fifteen traps were set for 24 hours and checked every 12 hours.

Captured females were weighed, measured (± 1mm; culmen, total tarsus, head, and wing chord) and approximately 1.0 - 1.5ml bood was taken from the tarsal or the brachial vein after dosing each bird with a single intragastric dose of deuterated water (*Appendix A*). Blood was collected into heparinzed tubes (BD Vacutainer, Franklin Lakes, NJ) using a winged infusion set (21 gauge needle, 30.5cm tubing, BD Vacutainer). Samples were kept cool for up to 2 hours until plasma and cells were separated by centrifugation at 3,000 x *g*. Plasma and cells were transferred to separate cryovials and stored at -20 °C. Females were marked with standard USFWS leg bands, an individually coded nasal disc, and a 9g prong-and-glue style transmitter (Pietz et al. 1995; Advanced Telemetry Systems, Isanti, MN). Unpaired males were banded and immediately released. Males that we suspected were paired were held in mesh bags or small plastic pet carriers until they could be released with their respective mates.

Radio-marked females were located 1-2 times per day throughout the breeding season (22 May -24 July, 2007; 17 May - 30 July, 2008) to determine breeding status (e.g. nesting, renesting or apparently not nesting). Birds that could not be located in the

immediate study area were monitored twice weekly via fixed-wing aircraft (Husky, Aviat Aircraft, Inc., Afton, WY). Birds that were located on land (i.e. suspected nesting) during aerial tracking were subsequently located by searching on foot using hand-held antennas. As part of long-term monitoring efforts, we revisited nests of marked birds throughout the breeding season to determine clutch size, nest fate and other reproductive parameters (for details, see *Appendix B*).

Laboratory Analysis

Carcass composition & follicle analysis

We thawed frozen carcasses of collected birds over night in a refrigerator. We measured whole body mass (± 1g) and the following morphological lengths (± 1mm): culmen, total tarsus, head, and wing chord. Feather mass was determined as the difference in mass between whole and plucked carcasses. We dissected and weighed (± 0.01g) the liver and flight muscles (pectoralis and supracoracoideus muscles). Visceral adipose was recorded as the combined weight of the abdominal fat pad and all disectable adipose in the messenteries surrounding the digestive tract. We weighed the digestive tract full and empty to estimate the mass of digesta by difference. Samples of the following components from female carcasses were removed and saved for separate chemical analyses: esophageal contents, liver, visceral adipose and pectoral muscle. The remainder of the carcass was reweighed and ground in a meat grinder using a 4.5mm seive (General Slicing, Murfreesboro, TN). Water content of the liver and ground carcass was determined by freeze-drying to constant mass. Dry samples were ground to a homogenous mixture in a coffee grinder (Kitchen Aid, St. Joseph, MI). Nitrogen content of dried whole carcass and liver was determined using an elemental analyzer (LECO Corporation, St. Joseph, MI). Lipids in liver and carcass were extracted with petroleum ether (Fisher Scientific, Fair Lawn, NJ) in a modified Soxhlet apparatus (Tecator, Höganäs, Sweden). We determined mineral ash content of carcasses by completely combusting a sample in a muffle furnace (Barnstead International, Dubuque, IA) for 8 hours at 550°C.

Stored reproductive tissues were examined under a 6.4-16.0x dissecting microscope (Bausch and Lomb, Rochester, NY) and follicles were classified as undeveloped (without visible yolk), pre-ovulatory (containing visible yolk), post-ovulatory, or atretic by the method of Esler (1994). We measured the diameter of pre-ovulatory follicles (POF) along the plane of the stigma (± 0.1mm) using a calipers. Tissues were rinsed in distilled water to remove surface debris and dried at 50°C to constant mass in a fan-forced oven. Dried POF were weighed to the nearest 0.01 g. Female reproductive status was classified as Rapid Follicle Growth (RFG) or Non-RFG following Esler (1994). RFG was defined as the stage when egg follicles begin to accumulate yolk (Johnson 2000). By this method, females with at least one POF with a dry mass $\geq$ 0.15g were classified as RFG.

We measured the mass of lipid committed to reproduction (reproductive lipid) by summing lipid content of dried POFs and estimated lipid in laid eggs. Lipid content of POFs was measured by lipid extraction as above for whole carcass. Because some POFs were too small to measure lipids accurately (n = 37) we used average lipid content of twenty POFs (mean = 56 ± 5%. [SE] of dry mass). The dry oviduct and remaining undeveloped follicles were assumed to be all protein and, therefore, not included in analysis (Alisauskas et al. 1990). Lipid content of laid eggs was calculated by counting the number of post-ovulatory follicles and multiplying by 6.82g, which is the estimated lipid content of whole scaup eggs (Afton and Ankney 1991).

Yolk precursor assays

Blood plasma samples (n = 34 and 37 from captured birds in 2007 and 2008, respectively and 6 from 2008 collected birds) were sent to the Alaska SeaLife Center (Seward, AK) for analysis of Very Low Density Lipoprotein (VLDL). VLDL concentrations peak in the blood when birds enter RFG and remain elevated throughout the laying period (Challenger et al. 2001; Gorman et al. 2009). VLDL concentrations have been used to determine RFG status for scaup (e.g., Martin et al. 2009) and other ducks (Safine 2005; Bond et al. 2008; Gorman et al. 2009; Martin et al. 2009). Plasma samples were assayed for total VLDL using a SpectraMax Plus^{384} plate reader (Molecular

Devices, Sunnyvale, CA) at 540nm and following the method of Mitchell and Carlisle (1991). Captured birds were classified as RFG if VLDL concentrations were ≥ 5.3 mmol/L (Gorman et al. 2009), an oviductal egg was palpated during capture, or the bird was subsequently found on a nest using telemetry observations.

Statistical Analysis

Samples of visceral adipose and pectoral muscle were removed from carcasses so masses of these components were added back for analysis. Visceral adipose samples were assumed to be mostly lipid and, therefore, used to index lipid reserves. Lipid content of dry pectoral muscle samples were measured directly and the remainder of the sample was assumed to be all protein. Total nitrogen in carcasses and livers was expressed as crude protein by multiplying nitrogen content by 6.25 (Robbins 2001). Ash-free lean (AFL) content of carcasses was calculated by subtracting lipid and ash mass from mass of dry matter. We used AFL mass to evaluate differences in protein content in all three years because crude protein measures were unavailable for birds collected in 1991. Due to small mass of livers we could not perform all analyses on liver tissues; we therefore assumed that ash content of liver was 4% of liver mass (P. Barboza, unpublished data) for calculations of AFL.

Statistical analyses were conducted using SYSTAT 10.2 (Systat Software Inc. 2002). We conducted principal components analysis on three morphological measures (culmen, tarus, and wing chord). The first principal component (PC1) was used as an index of structural body size (Sedinger et al. 1997; Mason et al. 2006). In all models, collection date was expressed as Julian day, which was standardized for leap year. We selected among competing models using $\alpha = 0.05$ as a criterion for statistical significance and, when necessary, divided for multiple comparisons using Bonferonni's adjustment. Masses of body components are presented as adjusted least squares means (g ± SE).

We examined the effects of RFG status (RFG or non-RFG), year and their interaction (RFG status*year) on body component mass (fresh body, dry matter, AFL, lipid and ash) using analysis of covariance (ANCOVA) while controlling for the effects of structural size (PC1) and collection date. To examine long-term temporal variation,

we ran a separate model to examine the effects of study (1991 or 2007/2008) and study*RFG status. In addition, we examined temporal trends in component masses using linear regression with year as a continuous independent variable. For this analysis, we corrected body component masses for structural body size (PC1). We did not adjust for Julian date because it was not significant for any components in any year ($p \geq 0.076$). We ran a linear regression for each body component against PC1 values to get residual values, then regressed residuals against year to examine patterns of mass change over time.

We used ANCOVA to examine the effects of RFG status, year and their interaction on additional body component masses (dry breast muscle, crude protein, visceral adipose, reproductive tract, liver dry matter, liver lipid and liver crude protein) measured for 2007 and 2008 collected birds. Because we were interested in examining the effects of reproductive investment on mass of total body lipid, we ran a logistic regression model for total body lipid that included the effects of reproductive lipid and year*reproductive lipid.

We ran a separate ANCOVA model for birds captured in 2007 and 2008 to examine the effects of year, RFG status and their interaction on mass of females.

Finally, we examined differences in fresh whole body mass of collected and captured birds in 2007-08 using ANCOVA. We included the effects of capture method, year and capture method*year but we did not include RFG status in this model because reproduction was assessed using different methods for collected and captured birds (see Methods).

RESULTS

We captured 34 females in 2007 and 37 females in 2008. Oviductal eggs were detected in three birds in 2007 and in one bird in 2008. Nesting of radiomarked birds was observed for three individuals in 2007 and for three birds in 2008. Yolk precursor analysis indicated that 8 females (21%) had entered RFG at the time of capture in 2007 and two (5%) had entered RFG in 2008. Yolk precursors correctly classified all females

that held an oviductal egg as entering RFG, but only classified one nesting bird as entering RFG.

We collected 18 females in 2007 and 28 females in 2008. The range of collection dates in 1991 was wider (8 May – 8 June; 31 days) than in either 2007 (29 May – 31 May; 3 days) or 2008 (27 May – 30 May; 3 days). One bird was excluded from analysis in 2007 due to extensive damage of body components from the shot. The proportion of birds classified as RFG using follicle examination methods ranged from 11 of 28 (39%) females in 2008, 12 of 18 (66%) in 2007 and 24 of 33 (73%) females in 1991. Yolk precursor analysis of a small sample (n = 6) of females collected in 2008 indicated some misclassification because precursor levels classified no birds as RFG at the time of collection even though enlarged follicles were dissected from two birds.

Arithmetic mean values (± SE) of morphometric measures and body component masses for captured, collected and captive scaup are reported in Table 1 and Table 2 (see *Appendix A* for a discussion of the use of males). All PC1 variable loadings were positive and ranged from 0.41 to 0.82. PC1 explained 48% of the total variation in morphological measures.

ANCOVA results indicated that all models that included an effect of year also included an effect of study. This indicates that yearly variation was sufficient to describe changes in body component masses and, therefore, we excluded the effect of study from further discussion.

We found no evidence for a temporal decline in body condition for birds collected in 1991, 2007 and 2008. In addition, reproductive status was associated with greater body condition as fresh mass and lipid mass were significantly larger in RFG than non-RFG females (Table 3, Fig 2). Models for dry matter, AFL and ash included a significant effect of year (Table 3) but regression analysis indicated no trend in mass of any component ($p \geq 0.098$). Though we found a large difference in the mean lipid mass (20g) between birds collected in 1991 and 2008 (Table 4), differences were not statistically significant (Table 3). We also found no effect of year on fresh body mass.

RFG status had a significant effect on fresh body mass and total body lipid (Table 3, Fig 2). Females in RFG were 80g heavier and contained 18g more lipid than birds classified as non-RFG. AFL and ash mass did not differ between RFG and non-RFG females (Table 3).

Comparisons between body components for birds collected in 2007 and 2008 support conclusions for birds in all years (Table 5). Overall, we found that year had a significant effect on measures of protein (breast muscle and liver crude protein; $p \leq 0.015$) but not lipid (visceral adipose and liver lipid; $p \geq 0.120$). In addition, we found that RFG status had significant effects on measures of lipid (visceral adipose and liver lipid; p = 0.0.19; Table 5): RFG females had 3g more visceral adipose and 0.2g more liver lipid. The model for crude protein was the only model that included a significant effect of RFG status*year (p = 0.007). This suggests that differences in total body protein stores of RFG and non-RFG birds vary between years. The mass of the reproductive tract did not vary between years after accounting for the expected variation in ovarian mass between RFG and non-RFG birds (Table 5).

Examination of the effects of reproductive investment (reproductive lipid) on mass of total body lipid indicate no significant effect (F = 1.436, p = 0.244), although the slope of the regression line was slightly negative (intercept = 77.95, slope = - 0.981, R^2 = 0.064). This suggests a small decline in body lipid as birds commit to reproduction.

ANCOVA results indicated a significant effect of breeding status (df = 1, F = 26.75, $p < 0.001$) and year (df = 1, F = 8.753, p = 0.004) on body mass of captured birds. LS mean values indicate that RFG (n = 55) females were 74g heavier than non-RFG (n = 13) females (716 ± 15g and 643 ± 7g, respectively) and females captured in 2007 (n = 33) were 39.8g heavier than those captured in 2008 (n = 38; 677± 10g and 637 ± 9g, respectively).

Comparisons of captured and collected birds indicate a trapping bias similar to that found in other studies (Weatherhead and Greenwood 1981; Fast et al. 2008). Birds captured using decoy traps were 89 g lighter (657 ± 7, n = 71) than collected birds (746 ± 9, n = 45; $p < 0.001$). Also, the proportion of females in RFG was lower in captured

birds even though the trapping period (16 May – 4 June) was longer and later than the collection period (27 May – 31 May) in both years. Variation in the proportion of females in RFG may be related to methodological differences in determining RFG status (yolk precursor analysis vs. follicle examination).

DISCUSSION

Temporal Changes in Body Condition

We tested the general hypothesis that continental declines in the population of lesser scaup over the last three decades were related to changes in the body condition of birds on their breeding grounds. Our first objective was to determine if a long-term trend in body condition was evident for female scaup breeding in the boreal forest of Alaska between 1991 and 2007-2008. Our results show no support for a long-term decline in any body components (including body mass, protein, lipid and mineral ash). In models that included a significant effect of study, year was also significant, (for models of AFL, dry matter and mineral ash). This suggests that mass variation of these components may be explained by inter-annual fluctuations rather than long-term shifts. Although our specific study included only one historical year for comparison our results are consistent with findings from other populations in the boreal forest (DeVink et al. 2008).

We were particularly interested in changes in mean body mass and lipid mass because long-term declines in these measures have been documented on the Mississippi Flyway (Anteau and Afton 2004) and lipid reserves maybe be particularly important for breeding females (Afton and Ankney 1991; Esler et al. 2001). However, we found no annual change in body mass or any measures of lipid. This lack of a decline on boreal forest breeding grounds in the face of significant declines on Midwestern migratory routes suggests that birds are able to recover lost nutrients on northern staging or breeding areas. Although we would expect scaup breeding in the boreal forest to incur large energetic costs during migration to Alaska, mean body mass and lipid mass of birds collected during migration in Manitoba and Minnesota in 2000 and 2001 (Anteau and Afton 2004) were very similar to our estimates for birds collected in Alaska in 2007 and

2008 (Midwest: body mass = ~770g, lipid = ~70g; Alaska: body mass = 753g, lipid = 66g). Alhough we do not know the origin of birds breeding on the YFNWR, little differences in mean mass of lipid between sites suggests that birds may be able to maintain or recover lost nutrients prior to breeding. Also, in a study of captive lesser scaup, Martin (2007) concluded that, given sufficient food resources, birds were able to recover quickly from a mass loss comparable to what has been observed in the Midwest. Martin (2007) estimated that fasted birds could recover from a 65g mass loss in 3-5 days on a diet of midges (Chironomidae) and amphipods (*Gammarus* and *Hyallela* spp). Birds spend approximately three to four weeks on breeding areas before nest initiation (Belrose 1980; Austin et al. 1998) and, therefore, may be able to recover lost nutrients prior to initiation of breeding.

A bird's ability to recover from mass losses sustained on migration depends on an adequate food supply. Although declines in forage quality (Anteau and Afton 2008b) and quantity (Anteau and Afton 2008a), have been documented in the Midwestern US and Canada, information in boreal forest wetlands are lacking. However, some evidence exists for long-term changes in food web structure in wetlands in the YFNWR where scaup breed (Corcoran et al. 2009). Increased temperatures may alter seasonal patterns of insect emergence in the arctic (Hodkinson et al. 1998) and some suggest that changes in freshwater biological communities have already occurred (Schindler 1997). If wetland communities change, food declines may force birds to rely on lower-quality diets or smaller prey that require more foraging effort and, consequently, increase the variation in daily food intakes. In addition, shifts in invertebrate hatching events may cause birds to miss peak food abundance. Indeed, peak hatching dates of diving duck broods often coincides with peak abundance of invertebrates (Bartonek and Hickey 1969). A mismatch in timing of these events could impact duckling growth and survival. A study of free-living blue tits (*Parus caeruleus*) in Europe found that mass of 14-day-old nestlings in a "mismatched" population was lower than in a matched population (Thomas et al. 2001). Consequently, scaup unable to adjust timing of breeding in response to these changes (i.e., if they rely on a fixed cue such as photoperiod) may experience reduced

reproductive output or survival. We concur with DeVink et al. (2008) that future research should examine a "mismatch hypothesis."

Poor condition or nutritional stress experienced along migratory routes may impact breeding efforts if birds commit to reproduction early or need to reach a nutrient threshold for initiation of breeding. Unreliable or insufficient food sources on migratory stopover areas and breeding grounds could cause birds to abandon breeding efforts or delay breeding, despite adequate body condition. Barboza and Jorde (2002) found that captive black ducks (*Anas rubripes*) fed intermittently delayed egg production, despite having greater body mass than birds with reliable food supplies. If scaup respond similarly, and habitat conditions have declined, birds may not commit to reproduction despite relatively good body condition upon arrival to the breeding grounds. Interactions between year and RFG status for models of body protein content may reflect increased variance in foraging on the breeding grounds (Table 3). During a fasting period, scaup mobilize the large reserve of protein in breast muscle more readily than either American Wigeon (*Anas americana*) or Northern Shovelers (*Anas clypeata*) consuming the same diet (Martin 2007). Greater variation in body protein may reflect foraging variation for scaup if restoring lipid mass is a priority during refeeding periods.

In addition, some evidence suggests that scaup may not breed every year. Scaup are relatively long-lived ducks that may not breed until 2-3 years of age (Austin et al. 1998). Estimates of breeding probability on boreal forest breeding areas are much lower than predicted (Martin et al. 2009, this study) and suggests that scaup may trade reproduction in one year for ensured survival into the next. Inadequate nutritional conditions experienced by females while en route to boreal forest breeding grounds could trigger a 'non-breeding' response that may be sustained despite an adequate food supply on the breeding grounds and/or a sufficient recovery of body condition. Thresholds of lipids stores for survival may vary annually with food supplies and environmental demands during migration, which may increase the threshold for reproduction and thus delay nesting on arrival (Barboza et al. 2009).

Scaup may also delay RFG initiation in response to poor conditions experienced during migration. DeVink et al. (2008) found that scaup in boreal Canada initiated RFG later than sympatric Ring-necked Ducks (*Aythya collaris*), despite having similar nutrient levels. However, they also noted that mean nest initiation date for scaup have not changed at the same site in the past 20 years. We note the same pattern on the YFNWR: median nest initiation dates reported in 1989-1991 were 3 June (n = 5), 8 June (n = 6) and 19 June (n = 18), respectively (Grand 1995) median dates in this study were 3 June, 2007 (n = 71) and 8 June, 2008 (n = 41).

Body Condition and Reproductive Status

Our second objective was to examine the relationship between reproductive status and body condition of female scaup. We found little evidence that protein limits reproduction in scaup, which is consistent with other work (Afton and Ankney 1991; Esler et al. 2001; DeVink et al. 2008; Gorman et al. 2008). Scaup feed mainly on high protein, low lipid foods such as amphipods and snails (*Gastropoda* spp.) and, therefore, are probably not protein limited. In addition, protein stores in the body are large (breast muscle, legs, gizzard) relative to lipid stores yet birds must commit approximately equal amounts of protein and lipid to eggs (Afton and Ankney 1991). Therefore, we focus our discussion on lipid reserves.

We expected that females that had entered RFG at the time of collection or capture would weigh more and carry more nutrients important for reproduction. Indeed, we found that mean body mass and lipid mass were significantly greater in collected females that had entered RFG. Low body mass of decoy-trapped females was also consistent with a lower incidence of RFG than collected birds. However, differences in reproductive status between collected and trapped birds may partially be the result of different methods used to assess RFG status (YP levels in captured birds, follicle examination in collected birds). YP methods are likely negatively biased (Gorman et al. 2009) because the length of time that VLDL levels are elevated (Safine 2005; Gorman et al. 2009) is shorter than the length of time that birds remain in RFG (>30 days; Martin et al. 2009; DeGroot, unpublished data).

Differences in lipid stores between RFG and non-RFG females have been identified for other waterfowl species (Alisauskas and Ankney 1994; Devries et al. 2008) and are consistent with a lipid limitation hypothesis suggested by others for Lesser (Afton and Ankney 1991; Esler et al. 2001) and Greater Scaup (Gorman et al. 2008). Such limitations may have consequences for breeding. Females with small lipid stores may be at a reproductive disadvantage and breed later, produce smaller clutches or lay smaller eggs (Alisauskas and Ankney 1992; Sedinger et al. 1995). Smaller lipid stores may also decrease incubation constancy and, therefore, nest success. Lipid stores may be especially important at northern latitudes, where thermoregulatory costs may be higher and the breeding season shorter. However, work comparing nutrient reserve dynamics of sympatric populations of waterfowl breeding in both subarctic and more temperate latitudes have mixed results (MacCluskie and Sedinger 2000; Esler et al. 2001).

Our data show large variation in body condition (Table 4) and includes several individuals for which body condition doesn't match expected reproductive status. In other words, many females with relatively small lipid reserves were in RFG at the time of collection while many non-RFG females appeared to have sufficient lipid reserves for follicle development, but had not initiated RFG. Body lipid influences egg laying in lesser scaup on the YFNWR: birds with less fat lay smaller eggs later in the season (Esler et al. 2001). However, we did not find a significant relationship between mass of body lipid and total lipid in follicles. This indicates that some females in 2007 and 2008 initiated RFG despite relatively poor condition. We interpret this to mean that females may rely upon body stores as well as local food sources for egg production. Indeed, DeGroot (2011) used a stable isotope approach to examine nutrient allocation strategies in scaup and found evidence for use of both endogenous and exogenous sources of lipid for production of eggs. This suggests that, although body lipids are important for reproduction, boreal forest prey also provide significant sources of nutrients. This provides further evidence that body condition, although important, may not be the only constraint on reproduction as previously thought.

The allocation of body lipid to reproduction implies a capital breeding strategy whereby birds use nutrient "capital" to meet the demands of egg production (Drent and Daan 1980). This strategy has been widely accepted in the literature for large-bodied birds nesting at high latitudes that may arrive on breeding grounds prior to the pulse in food availability (Drent and Daan 1980; Ankney 1984; Parker and Holm 1990). Endogenous lipid stores have been shown to be important for RFG initiation (Esler et al. 2006; Gorman et al. 2008; this study) and incubation (Afton and Ankney 1991) for scaup. However, we caution against underestimating the importance of local food resources. Other work on arctic and sub-arctic nesting birds (MacCluskie and Sedinger 2000; Klaassen et al. 2001; Gauthier et al. 2003), including greater scaup (Gorman et al. 2008), suggests that local food sources contribute large portions of nutrients to eggs. Specifically, work by DeGroot (2011) highlights the importance of breeding grounds for egg formation for this population of lesser scaup.

Variation in our data also showed that many females with relatively large lipid depots had not initiated RFG at the time of collection, including one female with the largest lipid mass (160g). This suggests that factors other than nutrition may be influencing breeding propensity in scaup. Macroexamination of follicles from a sample of post-breeding females (n = 20) collected at the Canvasback and Long Lake sites on 14-17 July, 2008 showed that only seven females had visible evidence of post-ovulatory follicles and only three of those had a brood patch. This provides additional support for low breeding propensity in this population first discussed in Martin et al. (2009).

We acknowledge that timing of collections may have influenced our sample. Collection windows were short (3-4 days) in 2007 and 2008 and our results may underestimate the proportion of RFG females in the population. In fact, mean RFG initiation dates of collected birds was 24 May in 2007 (± 0.09d) and 2008 (± 0.07d); mean RFG initiation dates calculated from a sample of nests monitored at the Long Lake study site in 2007 (n = 71) and 2008 (n = 41) indicated that most birds initiated on 31 May, 2007 (± 0.10d) and 3 June, 2008 (± 0.18d) which varied significantly from initiation dates of collected birds (t-test; 2007: $t = 2.84$, $p < 0.01$; 2008: $t = 4.80$, $p < 0.01$;

see *Appendix B*). This suggests that some collected birds may have entered RFG at a later date. Nonetheless, we make no assumptions about future breeding status of collected individuals and interpret our results based on RFG status at the time of collection.

Other aspects of scaup life history could have deleterious effects on reproductive output and much work done on scaup in Alaska has demonstrated relatively poor reproductive parameters. For example, nest success for scaup in Alaska is variable, but likely too low to support viable populations (Walker et al. 2005; Corcoran et al. 2007). Also, although there is little evidence of harmful levels of environmental contaminants in scaup (Custer et al. 2003; Fox et al. 2005; Petrie et al. 2007) elevated levels of strontium have been measured in scaup egg shells collected on the YFNWR and were negatively correlated with egg shell thickness (Matz and Rocque 2007). Reduced structural integrity of eggs may influence reproductive output similar to the effects of DDE on raptors (Hickey and Anderson 1968). In addition, duckling survival in interior Alaska is among the lowest reported for ducks nesting in northern regions and may cause poor recruitment (Walker and Lindberg 2005; Corcoran et al. 2007).

Although concern for reduced reproductive output in scaup is warranted, a more directed focus on factors that strongly influence scaup population dynamics is necessary. Notably, Koons et al. (2006) demonstrated that the population growth rate (λ) of lesser scaup is most sensitive to changes in the survival rate of females during the breeding and non-breeding seasons. Although λ was moderately sensitive to changes in nest success, duckling survival and juvenile survival, λ was most sensitive to changes in adult survival. These results for scaup are more similar to estimates in Emperor geese (*Chen canagica*; most sensitive to changes in survival; Schmutz et al. 1997) than Mallards (*Anas platyrynchos*; most sensitive to changes in nest success; Hoekman et al. 2002) and suggest that scaup may be more k-selected than previously thought. Compared to many dabbling duck species, scaup are relatively long-lived, late breeders (Austin et al. 1998) and many reproductive parameters suggest a more k-selected life-history such as low breeding probability (Martin et al. 2009), relatively small clutch sizes (Ankney et al.

1991) and low incidence of renesting (Austin et al. 1998). Life-history strategies of scaup may be more similar to large-bodied sea ducks such as eiders and scoters. Although this idea is theoretical and the line between k- and r-selected waterfowl species is a blurry one, this distinction could potentially influence management decisions and resource direction. Therefore, it is important that future work consider population-level approaches to scaup research and management.

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APPENDICES

Appendix A. Using Deuterated Water to Estimate Body Composition

We measured body condition of captured scaup by dilution of deuterated water (D_2O). Methods for dosing and blood sampling follow (Barboza and Jorde 2001b). Each animal was weighed (± 1g) and given a single intragastric dose (8 French x 54cm infant feeding tube) of approximately 1.5g deuterated water (D_2O; Aldrich Chemical, Milwaukee, WI). Doses of D_2O were previously measured in the laboratory (± 0.0001g) and sealed with a stopcock for use in the field. Prior to use, mass of dose syringes were checked using a portable scale (± 0.0001g) to ensure no dose was lost in transport. After dose administration, birds were kept cool and held in a small plastic pet carrier to allow equilibration of the D_2O dose. After 90 minutes a sample of blood was collected as described above. Birds were then measured and marked as described in Methods.

In addition, unpaired males (n = 10 each year) captured in decoy traps were used to validate the D_2O method. Males were weighed, dosed with D_2O, and bled following the protocol for females. After bleeding, males were euthanized with Isofluorane (Halocarbon Laboratories, River Edge, NJ), wrapped in plastic, double-bagged, and stored frozen. In 2007, we also euthanized five captive male scaup for validation.

In the lab, blood plasma samples (20μL) were thawed and diluted 1:200 with distilled water for isotopic analysis of water vapor at equilibrium in the presence of platinum-on-alumina as a catalyst. Ratios of deuterium (^2H) to hydrogen (^1H) were deteremined in a mass spectrometer (PDZ Europa GEO 20-20 Isotope Ration Mass Spectrometer with Gilson Autosampler, PDZ Europa, Ltd., Crewe, Cheshire, UK) and reported in delta (δ) notation that was expressed in parts per thousand (‰) relative to Vienna Standard Mean Ocean Water ($^2H/^1H$ ratio = 0.00015576). Analytical error was estimated to be ± 0.18% by running standards concurrently with samples. Samples were assayed in triplicate with a mean coefficient of variation of 1.5%. The concentration (F) of deuterium in total hydrogen follows Barboza and Jorde (2001a) and the following equation:

$$F = \frac{\left[(\delta/1000)+1\right]}{\left\{(1/0.00015576)+\left[(\delta/1000)+1\right]\right\}}$$

This concentration was calibrated against gravimetric standards of D_2O diluted with distilled water. Concentrations of D_2O in undosed birds (n=16) were used to determine background levels that were subsequently subtracted from levels in dosed birds. We calculated total water space using the deuterium dose (g D_2O) divided by the D_2O concentration at equilibration (g D2O g-1 water; Barboza and Jorde 2001b) calculated body composition of birds using total body mass and relationships between water space, lean mass, and lipid mass for 26 male Lesser Scaup collected for validation (Table 1). We used the following equations to calculate body composition of pre-breeding female lesser scaup:

Feather mass = 0.081*body mass

Digesta mass = 0.026*body mass

Net water space = [(water space – 30.27)/0.61] – (0.642*digesta mass)

Empty body mass = body mass – digesta mass – feather mass

Lean body mass = net water space/0.7079

Lipid mass = empty body mass – lean body mass

Standard errors for estimates of lean body mass and lipid mass were 15g and 16g, respectively.

Body composition estimates of scaup measured by water dilution were variable and not reliable. Lean mass estimates from water dilution of 26 male scaup differed from the direct measure by up to 39% (range = 0-39%, mean = 12%, SE = 2%, CV = 84%). Estimates of lean mass from water dilution of 82 females were 45-146% of the estimated empty body (mean = 96%, SE = 3%, CV = 23%). Body condition estimates were unreliable because dose rates were probably too low (1.5 g kg^{-1}) and lower than those

used previously for captive scaup in winter ($3g \cdot kg^{-1}$; Martin 2007). Poor equilibration of the dose may be related to high respiration rates of birds captured in spring when stress responses maybe higher than those in winter. Subsequent work with this species should use much higher dose rates (i.e., $3g\ kg^{-1}$) and may need to consider the use of sedatives to reduce the stress response (Barboza and Jorde 2001b; Martin 2007).

Appendix B. Monitoring Nests of Marked Birds

We monitored nests of marked birds to determine nest success and to gather additional information about scaup breeding ecology at the Long Lake study site. When a nest was found we recorded its location using a GPS unit and marked it with flagging placed 10m to the north. We candled a subset of eggs to determine incubation days (Weller 1956) and marked eggs with a permanent marker. We also recorded the following nest characteristics: distance to water (± 5m), habitat type (open meadow, floating bog mat, forest, shrub, or emergent vegetation), amount of downy feathers (none, some, abundant), number of eggs, and nest stage (laying [all eggs at 0 days incubation], incubating [at least one egg >0 days incubation], pipping [at least one egg with small cracks indicating the beginning of hatch], or hatching [at least one egg with large, open cracks or a visible duckling]). In order to reduce observer-influenced predation, observers concealed nests with down and vegetation before leaving the area.

We revisited nests every 5-7 days when females were known to be off the nest. On each visit, we recorded nest stage, nest condition (intact or failed), the number of new eggs, and we candled a subset of eggs to estimate incubation days. We suspected that a female had abandoned her nest if the estimate of incubation days had not changed from the previous visit and the female was consistently located off the nest during the visit interval. We returned to the nest after 2-4 days to confirm abandonment.

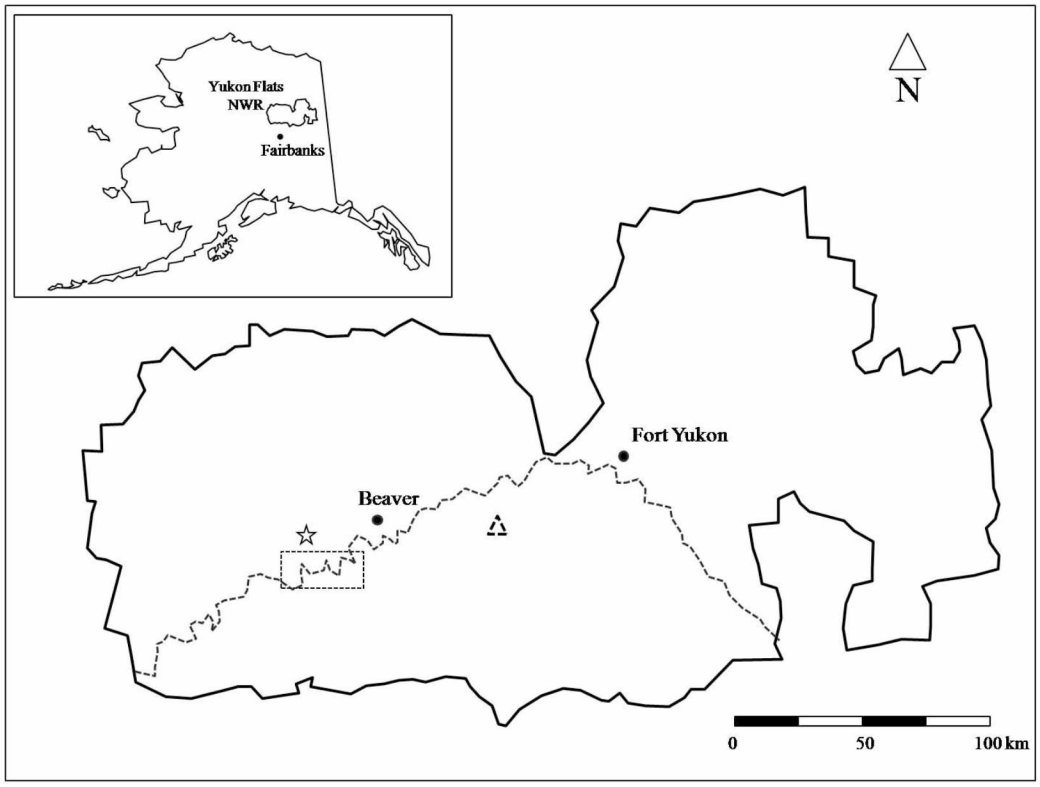

Fig 1. Sites on the Yukon Flats National Wildlife Refuge, Alaska where samples were collected. Female Lesser Scaup were collected in 2007 and 2008 at the Yukon River site (rectangle); additional females were collected in 2008 at the Canvasback Lake site (triangle). In 1991, females were collected at the Long Lake site (star). The Yukon River is indicated by a dashed line. Inset depicts the location of the study area in Alaska.

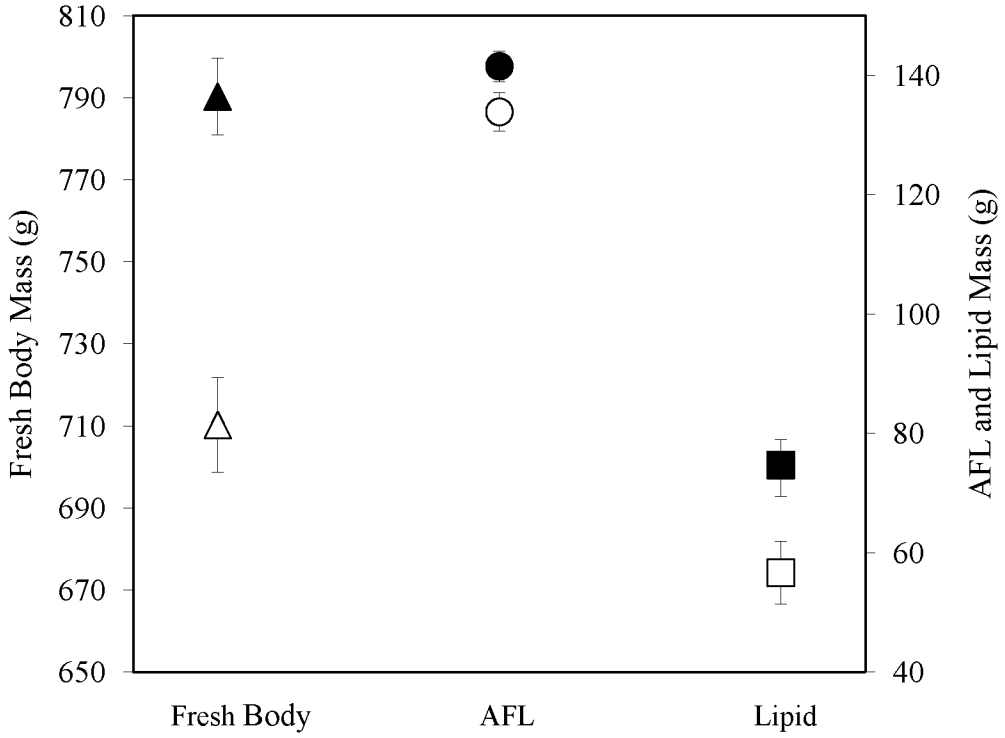

Fig 2. Least squares mean mass (g ± SE) of body components of pre-breeding female Lesser Scaup collected on the Yukon Flats National Wildlife Refuge, Alaska in 1991, 2007 and 2008. Values are corrected for structural body size (PC1) and collection date (Julian day; ANCOVA); 2008 was standardized for leap year. AFL = ash-free lean mass. Closed symbols represent birds in rapid follicle growth (RFG) and open symbols represent non-RFG females. Fresh body mass and lipid means are significantly different ($p \leq 0.013$); AFL means do not differ ($p = 0.079$).

Table 1. Arithmetic mean values (± SE) of measurements of pre-breeding Lesser Scaup collected on the Yukon Flats National Wildlife Refuge, Alaska in 2007 and 2008 and captive males raised at the University of Alaska Fairbanks. All masses are reported dry except fresh whole carcass, fresh liver and feathers. RFG = rapid follicle growth.

		Wild Females				Wild Males[1]		Captive Males
		RFG		Non-RFG				
		2007	2008	2007	2008	2007	2008	2008
		(n = 11)	(n = 11)	(n = 6)	(n = 17)	(n = 10)	(n = 11)	(n = 5)
Morphometrics (mm)	Culmen	39.9 ± 0.1	41.8 ± 0.1	40.6 ± 0.2	40.9 ± 0.1	40.2 ± 0.2	41.5 ± 0.1	41.6 ± 0.2
	Tarsus	42.2 ± 0.1	42.9 ± 0.1	42.5 ± 0.2	42.8 ± 0.1	43.8 ± 0.1	43.2 ± 0.2	45.6 ± 0.3
	Head	85.4 ± 0.1	87.2 ± 0.2	86.6 ± 0.3	86.2 ± 0.2	89.9 ± 0.3	89.3 ± 0.2	88.1 ± 0.3
	Wing chord	192 ± 0	194 ± 0	192 ± 1	192 ± 0	203 ± 0	202 ± 0	195 ± 1
Whole carcass (g)	Fresh	768 ± 5	788 ± 4	723 ± 9	717 ± 4	627.7 ± 4.0	620.7 ± 2.5	562.0 ± 2.7
	Dry matter	257.3 ± 2.0	263.6 ± 1.5	243.8 ± 7.9	228.2 ± 2.2	213.0 ± 2.2	216.2 ± 1.9	186.7 ± 4.4
	Crude protein	136.4 ± 1.0	148.1 ± 1.0	146.5 ± 3.2	130.5 ± 0.8	140.6 ± 1.7	133.3 ± 0.7	112.1 ± 0.8
	Lipid	61.8 ± 2.3	85.2 ± 1.1	47.9 ± 6.2	60.1 ± 2.0	30.8 ± 2.1	50.0 ± 1.8	27.4 ± 3.0
	Ash	31.3 ± 0.3	33.1 ± 0.4	29.8 ± 0.5	32.2 ± 0.2	32.0 ± 0.3	34.2 ± 0.3	24.1 ± 0.4
Liver (g)	Fresh	24.0 ± 0.2	28.1 ± 0.3	24.3 ± 0.3	25.9 ± 0.3	22.3 ± 0.4	25.4 ± 0.3	13.6 ± 0.6
	Dry matter	7.0 ± 0.1	8.2 ± 0.1	7.2 ± 0.1	7.4 ± 0.1	6.0 ± 0.1	6.7 ± 0.1	3.7 ± 0.2
	Crude protein	5.2 ± 0.0	6.2 ± 0.1	5.5 ± 0.0	5.6 ± 0.1	4.9 ± 0.1	5.7 ± 0.1	3.1 ± 0.1
	Lipid	0.9 ± 0.0	0.9 ± 0.0	0.8 ± 0.0	0.6 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.1 ± 0.0
	Ash[2]	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.1 ± 0.0
Feathers[3] (g)		129.1 ± 2.5	66.9 ± 0.9	133.8 ± 6.7	60.9 ± 0.7	42.6 ± 0.4	39.5 ± 0.3	29.4 ± 0.1
Flight muscles[4] (g)		38.2 ± 0.2	42.2 ± 0.3	36.5 ± 0.3	39.1 ± 0.2	35.7 ± 0.2	36.4 ± 0.2	31.9 ± 0.5
Visceral adipose[5] (g)		10.9 ± 0.3	9.5 ± 0.2	6.8 ± 0.5	7.4 ± 0.3	4.4 ± 0.2	5.4 ± 0.3	4.7 ± 0.5
Digesta[6] (g)		8.7 ± 0.2	10.4 ± 0.4	11.3 ± 0.8	8.7 ± 0.2	3.7 ± 0.1	3.6 ± 0.1	3.2 ± 0.2

[1]captured using live decoys

[2]estimated at 4% of liver dry matter

[3]feathers from 2007 females were very wet and masses likely include water

[4]includes pectoralis and supracoracoideous muscles

[5]combined mass of dissectable adipose from the abdominal fat pad and messentaries surrounding the intestines

[6]includes contents of esophagus, proventriculus, gizzard, intestines and cloaca

Table 2. Arithmetic mean values (± SE) of morphological measures and body mass of female Lesser Scaup captured on the Yukon Flats National Wildlife Refuge, Alaska in 2007 and 2008 using decoy traps. RFG = rapid follicle growth.

		RFG				Non-RFG			
		2007		2008		2007		2008	
		(n = 9)		(n = 4)		(n = 22)		(n = 34)	
Morphometrics (mm)	Culmen	39.8	± 0.2	39.3	± 0.5	39.9	± 0.1	40.0	± 0.0
	Tarsus	37.7	± 1.4	42.6	± 0.3	42.3	± 0.1	42.4	± 0.0
	Head	86.9	± 0.3	86.3	± 0.4	86.6	± 0.1	87.3	± 0.1
	Wing Chord	194	± 0	193	± 2	194	± 0	193	± 0
Body Mass (g)		718.4	± 5.5	709.8	± 11.7	665.7	± 2.7	626.7	± 1.1

Table 3. Results of ANCOVA models testing for the effects of RFG status, year, and their interaction on body components of pre-breeding female lesser scaup (n = 78) collected in 1991, 2007 and 2008 on the Yukon Flats National Wildlife Refuge, Alaska. All models are adjusted for structural body size (PC1) and collection date (Julian day); 2008 was standardized for leap year. All masses are reported dry except for fresh whole body; all components exclude feathers, digesta, and reproductive organs except fresh whole body. RFG = rapid follicle growth.

Model	Predictor	df	F		p	
Fresh whole body	RFG status	1	26.75	<	0.001	*
	Year	2	2.34		0.104	
	RFG status*year	2	1.15		0.324	
Dry matter	RFG status	1	8.93		0.004	*
	Year	2	5.24		0.008	*
	RFG status*year	2	0.85		0.432	
Ash-free lean	RFG status	1	3.18		0.079	
	Year	2	54.10	<	0.001	*
	RFG status*year	2	1.26		0.290	
Lipid	RFG status	1	6.47		0.013	*
	Year	2	2.19		0.120	
	RFG status*year	2	0.58		0.563	
Ash	RFG status	1	1.00		0.320	
	Year	2	4.14		0.020	*
	RFG status*year	2	0.55		0.581	

*statistically significant models ($p < 0.05$)

Table 4. Least squares mean mass (g ± SE) of body components of pre-breeding female Lesser Scaup (n = 78) collected in 1991, 2007 and 2008 from the Yukon Flats National Wildlife Refuge, Alaska. Values are adjusted for structural body size (PC1) and collection date (Julian day; ANCOVA); 2008 was standardized for leap year. Different superscripts denote significant ($p < 0.05$) component differences between years. All masses are reported dry unless noted. All components except fresh whole body exclude mass of feathers, digesta and reproductive organs.

	1991 (n = 33)		2007 (n = 17)		2008 (n = 28)	
Fresh whole body[1]	788.8 ± 16.9	a	747.9 ± 17.6	a	726.2 ± 17.2	a
Dry matter	213.5 ± 8.5	a	256.5 ± 8.8	b	247.9 ± 8.6	ab
Ash-free lean	126.1 ± 2.7	a	164.3 ± 2.8	b	137.1 ± 2.7	a
Lipid	59.7 ± 7.0	a	61.7 ± 7.2	a	79.5 ± 7.1	a
Ash	27.7 ± 0.7	a	30.4 ± 0.8	ab	31.3 ± 0.8	b
Breast muscle[2]			37.9 ± 0.7	a	40.3 ± 0.5	b
Body crude protein			143.9 ± 3.5		137.6 ± 2.6	
Visceral adipose[3]			9.3 ± 1.1		8.2 ± 0.8	
Liver dry matter			6.9 ± 0.3		7.8 ± 0.2	
Liver crude protein			5.2 ± 0.2	a	5.9 ± 0.1	b
Liver lipid			0.9 ± 0.1		0.7 ± 0.0	
Reproductive tract[4]			5.6 ± 1.5		2.7 ± 1.1	

[1] wet mass

[2] includes pectoralis and supracoracoideous muscles

[3] combined mass of dissectable adipose from the abdominal fat pad and mesentery surrounding the digestive tract

[4] ovarian tissue dissected above the cloaca to the infundibulum

Table 5. Results of ANCOVA models testing for the effects of RFG status, year and their interaction on body components of pre-breeding female Lesser Scaup (n = 45) collected in 2007 and 2008 on the Yukon Flats National Wildlife Refuge, Alaska. All models are adjusted for structural body size (PC1) and collection date (Julian day); 2008 was standardized for leap year. All masses are reported dry except for visceral adipose; all components exclude digesta and reproductive organs. RFG = rapid follicle growth.

Model	Predictor	df	F	p	
Breast muscle[1]	RFG status	1	8.19	0.007	*
	Year	1	6.53	0.015	*
	RFG status*year	1	0.10	0.755	
Crude protein	RFG status	1	0.66	0.420	
	Year	1	1.90	0.176	
	RFG status*year	1	7.97	0.007	*
Visceral adipose[2]	RFG status	1	5.98	0.019	*
	Year	1	0.58	0.452	
	RFG status*year	1	0.03	0.869	
Liver dry matter	RFG status	1	0.30	0.585	
	Year	1	4.38	0.043	*
	RFG status*year	1	0.84	0.365	
Liver crude protein	RFG status	1	0.23	0.635	
	Year	1	7.30	0.010	*
	RFG status*year	1	3.87	0.056	
Liver lipid	RFG status	1	5.95	0.019	*
	Year	1	1.71	0.198	
	RFG status*year	1	2.27	0.140	
Reproductive tract[3]	RFG status	1	11.47	0.002	*
	Year	1	2.13	0.153	
	RFG status*year	1	1.05	0.311	

[1]includes pectoralis and supracoracoideous muscles

[2]combined mass of dissectable adipose from the abdominal fat pad and intestinal messentaries

[3]ovarian tissue dissected above the cloaca to the infundibulum

*statistically significant models ($p < 0.05$)

CHAPTER 2. NUTRIENT ALLOCATION STRATEGIES OF LESSER SCAUP IN THE BOREAL FOREST OF ALASKA: AN ISOTOPIC ASSESSMENT USING $\delta^{13}C$ AND $\delta^{15}N$[1]

ABSTRACT

Continental populations of Lesser Scaup (*Aythya affinis*; hereafter, scaup), a medium-sized diving duck, have been declining since the early 1980's and declines are particularly pronounced in the boreal forest, where most scaup breed. One well-established hypothesis suggests that poor body condition of breeding females may explain reduced reproductive output. Therefore, it is important to understand how stored nutrients contribute to reproduction in scaup. Our objective was to use stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) to examine allocation of endogenous and exogenous fat and protein to egg yolk. In addition, we examined inter- and intra-female variation in egg yolk signatures. Pre-breeding female scaup were collected on the Yukon Flats National Wildlife Refuge, Alaska, USA, in 2007 and 2008 (n = 23 females and 55 yolks). We analyzed the isotope signatures of tissues (red blood cells, blood plasma, lipid-free muscle, lipid-free liver, abdominal lipid), egg follicles (lipid-free yolk) and local food items (amphipods; *Gammarus* and *Hyalella* spp.) to evaluate relative contributions of endogenous and exogenous carbon and nitrogen to yolks. We found that female scaup rely heavily on diet for production of protein in egg yolk, with little variation between years. The pattern for lipid was less clear, but scaup appeared to use a mixed capital-income strategy for commitment of C in lipid. Variation in isotope signatures among yolk follicles within the same female was small and indicated that scaup do not allocate nutrients differently to successive eggs. Our results highlight the importance of invertebrate prey on breeding areas for reproduction for scaup in the boreal forest.

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INTRODUCTION

Migratory birds employ a range of strategies for acquiring nutrients necessary to meet the demands of egg production. Typically, species are described as being somewhere along a continuum between body capital and dietary income. Strictly capital breeders use nutrients stored in the body as the primary input to eggs (Common Eider [*Somataria mollissima*], Parker and Holm 1990), whereas income breeders primarily use local food sources for reproduction (most passerines, Meijer and Drent 1999; Northern Shoveler [*Anas clypeata*], MacCluskie and Sedinger 2000; wading birds, Klaassen et al. 2001; Redhead [*Aythya americana*], Hobson et al. 2004; King Eider [*Somateria spectabilis*], Lawson 2006 and Oppel 2008; Harlequin Duck [*Histrionicus histrionicus*], Bond et al. 2007; Greater Scaup [*Aythya marila*], Gorman et al. 2008). However, many species fall somewhere in the middle of the continuum by using a mix of both capital and income (Canvasback [*Aythya valisineria*], Barzen and Serie 1990; Ruddy Duck [*Oxyura jamaicensis*], Alisauskas and Ankney 1994; Greater Snow Goose [*Chen caerulescens atlantica*], Gauthier et al. 2003; Barrow's Goldeneye [*Bucephala islandica*], Hobson et al. 2005; Long-tailed Duck [*Clangula hyemalis*], Lawson 2006; Emperor Goose [*Chen canagica*] and Black Brant [*Branta bernicla*], Schmutz et al. 2006).

As a taxa, waterfowl have been well studied with respect to nutrient investment in reproduction. Waterfowl are relatively large-bodied birds that invest proportionately large amounts of nutrients in energy-dense eggs (Alisauskas and Ankney 1992). For this reason, body condition of pre-breeding females has been thought to influence subsequent reproductive output (Alisauskas and Ankney 1992; Webster et al. 2002).

Conventional methods for measuring nutrient allocation in waterfowl have relied upon "mass balance" techniques that equate lipid and protein losses in the female with gains in the clutch (Ankney and MacInnes 1978; Sedinger et al. 1997; Esler et al. 2001). However, these methods cannot account for nutrients used to meet the female's energetic requirements for body maintenance, which may be substantial, especially for birds that expend significant amounts of energy capturing prey (i.e., diving ducks). Therefore,

conventional methods may overestimate endogenous nutrients used for reproduction (Gauthier et al. 2003).

Naturally occurring isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) have been used to examine patterns of nutrient investment in waterfowl (Gauthier et al. 2003; Hobson et al. 2004; Hobson et al. 2005; Lawson 2006; Schmutz et al. 2006; Bond et al. 2007). This approach uses distinct signatures in endogenous (body tissues) and exogenous (diets) sources of nutrients to estimate proportionate contributions to egg components. Consumer tissues reflect the assimilated markers from the diet in a time sequence that depends upon the level of food intake, assimilation efficiency, metabolic inter-conversion and the rate of turnover of body tissue (DeNiro and Epstein 1978, 1981; Karasov and Martinez del Rio 2007; Barboza et al. 2009). Differences in metabolism can be used to distinguish nutrients acquired over a long period in tissue with slow rates of turnover from those nutrients assimilated directly from the current diet in rapidly replaced tissues. For example, muscle and red blood cells have slow rates of $\delta^{13}C$ turnover in birds (half-life of 12.4 and 29.8 days, respectively), whereas liver (2.6 days) and blood plasma (2.9 days) turn over relatively quickly and better reflect recently incorporated nutrients (Hobson and Clark 1992a). Therefore, we can estimate proportionate input of each source to reproduction for species that occupy isotopically distinct habitats during breeding and wintering periods (i.e., marine vs. terrestrial environments; Hobson et al. 1997).

The combined populations of Lesser (*Aythya affinis*) and Greater (*A. marila*) Scaup have been declining across North America since the early 1980's (Afton and Anderson 2001; Zimpfer et al. 2009). Annual US Fish and Wildlife Service surveys show that scaup populations dropped by approximately 150,000 birds per year between 1978-1997, and are now 18% below the long term average (Zimpfer et al. 2009). These declines are of particular concern for lesser scaup (hereafter, scaup) that constitute up to 89% of the continental population (Belrose 1980).

Lesser scaup are long-distance migrants that winter in freshwater and marine environments of the US and Mexico and breed in a wide range of habitats, from the

prairie pothole region of the Midwest to the boreal forest of Canada and Alaska (Austin et al. 1998). One well-established hypothesis suggests that poor body condition of breeding females may explain reduced reproductive output (the spring condition hypothesis [SCH]; Austin et al. 2000; Afton and Anderson 2001; Anteau and Afton 2004). Therefore, it is important to understand nutrient reserve dynamics of scaup and the relative input of endogenous protein and lipid, presumably acquired from midcontinent staging areas, for subsequent deposition in eggs. Although conventional methods suggest boreal scaup use a mixed capital/income strategy (Esler et al. 2001) we employed stable isotope methods to assess investment of lipid and protein to egg yolks.

Our main objective was to examine allocation of endogenous and exogenous nutrients to egg yolk of lesser scaup. In addition, we examine isotopic variation of egg yolks developed within individuals (intra-clutch variation) and between individuals in the population (intraspecific variation).

Study Area

Our study took place on the Yukon Flats National Wildlife Refuge (YFNWR), located in interior Alaska, approximately 160 km north of the city of Fairbanks (Fig 1). The YFNWR encompasses approximately 36,500 km^2 of boreal forest bisected by the Yukon River. The landscape is classified as 48% wetland, much of it closed-basin (USFWS 1987). Lakes and ponds are surrounded by emergent vegetation (including bulrush [*Typha latifolia*]) and open meadows; upland habitats include stands of white spruce (*Picea glauca*), quaking aspen (*Populus tremuloides*), and paper birch (*Betula papyrifera*). Extensive wetlands support large numbers of breeding waterfowl. The YFNWR is home to the second largest concentration of breeding ducks in Alaska, with over 100,000 pairs counted each year (Conant and Groves 2005). Scaup (lesser and greater combined) are the most abundant duck on the YFNWR and this area has been regarded as an important breeding area for the continental population of lesser scaup (USFWS 1986).

METHODS

Field Collections

We collected paired female scaup from 29 to 31 May, 2007 and from 27 to 30 May, 2008, which is within the period of arrival and nest initiation on the YFNWR. Birds were killed with shotgun (12 gauge) or rifle (.222) without the aid of decoys (Weatherhead and Greenwood 1981; Fast et al. 2008). We collected birds at one site in 2007 (n = 18) and two sites in 2008 (n = 29); maximum distance between collection sites was 83 km (Fig 1). As soon as the carcass was retrieved, birds were weighed to the nearest 10g using a Pesola spring scale (Baar, Switzerland) and approximately 1ml of blood was collected directly from the ventricle of the heart with a 21 gauge needle and 2mL syringe (BD Vacutainer, Franklin Lakes, NJ). In 2008, blood samples were centrifuged at 3,000 x *g* to separate plasma and cells. The reproductive tract was dissected and stored in 10% formalin. Carcasses and blood samples were wrapped in two layers of plastic and two layers of freezer bags to retain moisture, then carcasses and blood were kept cool until they could be stored frozen, usually within 12 – 48h.

Diets of breeding scaup in Alaska are poorly understood. Therefore, we collected a variety of macroinvertebrate taxa common in YFNWR wetlands and known to be consumed by scaup in other regions (Afton and Hier 1991; Afton et al. 1991; Anteau and Afton 2006, 2008b) to represent scaup diet. We collected amphipods (*Gammarus* and *Hyalella* spp.) from five adjacent wetlands in May, June and July 2008. In June 2009 we collected amphipods, snails (*Gastropoda spp.*) and leeches (*Hyrudinea* spp). Invertebrates were captured with small nets and kept cool until they could be stored frozen within 12 hours.

Laboratory Analysis

Tissue, follicle and diet sampling

Stored reproductive tissues were examined under a 6.4-16.0 x dissecting microscope (Bausch and Lomb, Rochester, NY). Egg follicles were classified as undeveloped (without visible yolk), pre-ovulatory (containing visible yolk), post-

ovulatory, or atretic following Esler (1994). We measured the diameter of pre-ovulatory follicles (POF) along the plane of the stigma (± 0.1mm) using a calipers. All follicles and oviductal eggs were rinsed in distilled water to remove surface debris and dried to constant mass in a fan-forced oven at 50°C. Dried POF were weighed to the nearest 0.01g, ground to a homogenous mixture using mortar and pestle, and stored at room temperature. Females were classified as Rapid Follicle Growth (RFG) or Non-RFG following Esler (1994). RFG is defined as the stage when egg follicles begin to accumulate yolk (Johnson 2000). By this method, females were classified in RFG if the reproductive tract contained at least one POF with a dry mass $\geq$ 0.15g. We used 53 POFs, 2 oviductal eggs and 23 carcasses of RFG birds for analysis.

In the laboratory, we thawed frozen invertebrates and bird carcasses in a refrigerator over night. Snails were dissected using forceps to separate shells from soft bodies and shells were discarded. Leech, amphipod, and snail samples were then rinsed with distilled water and stored frozen. We removed esophageal contents, livers and 1g each of visceral adipose and breast muscle from each bird. Only breast muscle and esophageal samples were collected from non-RFG females (n = 24). We recorded identifiable food items in esophageal contents, then rinsed and dried samples to constant mass in a fan-forced oven at 50°C. Snail shells were not removed from esophageal samples because most snails were too small to effectively remove soft bodies and many shells were crushed. All frozen samples were lyophilized to constant mass then ground to a homogenous mixture with mortar and pestle (muscle, liver, yolk, invertebrates, red blood cells and plasma). We extracted lipids in petroleum ether (Fisher Scientific, Fair Lawn, NJ) from liver, egg follicles, adipose, breast muscle, amphipod and esophageal contents by using a modified Soxhlet apparatus (Tecator, Höganäs, Sweden). Lipid-free tissues were dried at 80°C overnight to remove solvents. Lipids were collected from adipose tissue during the Soxhlet procedure into 1.5g aluminum oxide (ICN Pharmaceuticals, Eschwege, Germany) for subsequent isotope analysis. Lipid content of liver and follicle was estimated by mass difference between whole and lipid-free samples.

Carbonates ($CaCO_3$) present in shells and exoskeletons of invertebrates can enrich $\delta^{13}C$ signatures (Bunn et al. 1995). Therefore, carbonates were removed from invertebrates by washing the dry sample in 0.1 N HCl for 1h to release CO_2 before decanting excess fluid and allowing the residue to dry for 24 hours under a fume hood at room temperature.

Stable Isotope Methods

Approximately 0.1-0.4 mg of untreated (hereafter, bulk) and lipid-free samples were weighed into tin capsules and analyzed for stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes using a continuous flow isotope-ratio mass spectrometer (model ThermoElectron Delta V Plus, Thermo Scientific, Waltham, MA) at the Alaska Stable Isotope Facility (Fairbanks, AK). Precision of measurements was determined by analyzing a peptone standard concurrently with samples and was determined to be ±0.02‰ and ±0.01‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively. We report results as ratios in δ-notation relative to Vienna PeeDee Belemnite for C and to atmospheric air for N according to the following equation:

$$\delta X = [(R_{sample}/R_{standard}-1)] * 1000$$

where X equals ^{13}C or ^{15}N and R equals the ratio of $^{13}C/^{12}C$ or $^{15}N/^{14}N$, respectively (Hobson 1995).

We calculated lipid $\delta^{13}C$ for follicles and livers by mass-balance using the approach of Lawson (2006) because most follicle yolks and livers were very small and, therefore, lipid proportions of these samples were difficult to extract and handle. By Lawson (2006),

$$\text{Lipid } \delta^{13}C = ([\delta^{13}C \text{ Bulk}] - [\delta^{13}C \text{ Lipid-free} * PP])/LP$$

where LP represents the lipid proportion of the material and PP represents the protein proportion. For yolks, we measured mean lipid content by extracting lipids from follicle samples weighing at least 0.5g (n = 20; LP = 55.7%). We estimated yolk protein

by calculating crude protein content as N content*6.25 (Robbins 2001; PP = 35.5%). Nitrogen content of samples was derived from mass spectrometer output. We calculated lipid $\delta^{13}C$ of livers separately for each individual because lipid content of livers varied (mean = 12.0%, SD = 0.03%, CV = 23.4%).

Statistical Analysis

Statistical analyses were conducted using SYSTAT 10.2 (Systat Software Inc. 2002). We used linear regression with $\alpha = 0.05$ as criterion for statistical significance. Values are presented as adjusted least squares means ± SE, unless noted.

We examined spatial, inter- and intra-annual variation within amphipod samples and between samples representing potential diet (i.e., amphipods, snails, leeches, esophageal contents and blood plasma) using ANOVA with Tukey's test for multiple comparisons. We tested for inter-annual variation in $\delta^{13}C$ and $\delta^{15}N$ signatures of all body components and differences in isotope signatures of muscle tissue between RFG and non-RFG females using two-sample t-tests. In addition, we evaluated isotopic differences between bulk and lipid-free $\delta^{13}C$ and $\delta^{15}N$ values of amphipods and $\delta^{15}N$ of follicles, liver and breast muscle separately with paired t-tests. We examined inter-and intra-female variation in isotope signatures and shifts in $\delta^{13}C$ and $\delta^{15}N$ values with increasing follicle size separately using linear regression.

RESULTS

Diet Items

We found significant variation in mean $\delta^{13}C$ and $\delta^{15}N$ values between potential food items (Table 1; $p < 0.01$). $\delta^{15}N$ signatures of amphipods, snails and esophageal contents did not differ significantly ($p \geq 0.57$). Leech $\delta^{15}N$ signatures were significantly enriched compared to amphipods and esophageal contents ($\geq 3.1‰$; $p \leq 0.02$), possibly reflecting the signatures of vertebrates on which leeches feed. Therefore, we excluded leeches from further analysis. $\delta^{13}C$ signatures did not differ between amphipods and snails ($p = 0.95$). However, esophageal contents were significantly enriched in $\delta^{13}C$

compared to amphipods and snails ($\geq$ 3.3‰; $p \leq 0.01$), probably due to the presence of carbonates in snail shells that remained in esophageal samples. Signatures of short-term blood plasma and liver were generally enriched compared to diet items by 2.0 – 3.2‰ for $\delta^{15}N$ and 1.1 - 4.7‰ for $\delta^{13}C$.

We selected amphipods to represent scaup died because amphipods are abundant on the Yukon Flats NWR (Corcoran et al. 2009) and are known to be an important food item for scaup in other parts of their range (Table 2, Afton and Hier 1991; Afton et al. 1991; Anteau and Afton 2006; Anteau and Afton 2008b). In addition, amphipods were found in the esophagus of most collected birds in this study.

Amphipod signatures varied by collection site (pond or lake; Table 1; ANOVA, $\delta^{13}C$: df = 4, F = 8.65, p = 0.003; $\delta^{15}N$: df = 4, F = 7.80, p = 0.004) but did not vary either between years or between collection periods within a year ($p > 0.45$ for $\delta^{13}C$ and $\delta^{15}N$).

We found no difference in $\delta^{13}C$ signatures between bulk amphipods and those lipid-extracted and washed with HCl (mean bulk $\delta^{13}C$ = -32.58‰, mean lipid-free $\delta^{13}C$ = -32.62, t = -0.22 , p = 0.83).

Lipid extraction influenced $\delta^{15}N$ signatures of amphipods, breast muscle and yolk, but did not affect liver. Lipid-free extracts of amphipods (9.28 vs. 8.70‰, t = 3.9, p = 0.002) and yolk (7.15 vs. 6.59‰, t = -7.40, $p < 0.001$) were significantly depleted in ^{15}N compared to bulk samples whereas lipid-free breast muscle was enriched (11.91 vs. 12.16‰, t = -8.34, $p < 0.001$) in comparison with bulk samples. We used bulk $\delta^{15}N$ values for all samples in subsequent analyses.

Body Components

Isotopic signatures of some body components varied by year ($p \leq 0.01$ for lipid-free $\delta^{13}C$, lipid $\delta^{13}C$ and $\delta^{15}N$ of yolk and $\delta^{15}N$ of liver) so we analyzed 2007 and 2008 separately for all components.

Mean muscle $\delta^{13}C$ and $\delta^{15}N$ signatures did not differ between RFG and non-RFG females ($p \geq 0.179$).

Coefficients of variation (CV) within body components were generally greater for C than for N: 9.0 - 34.6% for $\delta^{13}C$ in lipid, 8.5 - 23.6% for $\delta^{13}C$ in protein and 2.2 -

29.2% for $\delta^{15}N$. Isotopic variation between protein depots known to have short- (liver and plasma; 4.9 -23.6%) and long-term (muscle, blood cells; 6.5 – 22.6%) rates of isotopic turnover did not differ for either $\delta^{13}C$ or $\delta^{15}N$.

Patterns of isotopic enrichment and depletion between body components were consistent for $\delta^{15}N$ and $\delta^{13}C$ (protein and lipid; Fig 2 and Fig 3; Appendix A and Appendix B). As expected, short-term nutrient depots were generally more depleted than long-term pools. Yolk signatures were depleted in $\delta^{13}C$ and $\delta^{15}N$ compared to all other body components for both protein and lipid. In addition, $\delta^{15}N$ of muscle tissue was enriched above diet by approximately 2.7‰. This is within the expected range of diet-to-muscle fractionation for other vertebrates (Vanderklift and Ponsard 2003).

For body components sampled in both years (yolk, liver and muscle), 2008 signatures were always more enriched in $\delta^{15}N$ and depleted in $\delta^{13}C$. This suggests a consistent annual pattern of nutrient routing despite annual variation in isotopic signatures of tissues.

Yolk

Linear regression results indicated that isotope signatures of egg follicles did not change with increasing follicle mass (g dry matter) for either $\delta^{13}C$ or $\delta^{15}N$ ($p \geq 0.36$; Appendix C). In addition, variation in follicle $\delta^{15}N$ and $\delta^{13}C$ was small but slightly greater between females than within females (Appendix D). Intra-female CV's for five individuals with ≥ 4 developing follicles was 1.5% for carbon and 9.8% for nitrogen. In contrast, CV's between females were 8.5 and 13.2% for carbon and nitrogen, respectively. Similarly, intra-female CV's for $\delta^{13}C$ in yolk lipid equaled 4.6% compared to 9.3% between females.

DISCUSSION

Diet Items

Large variation in potential scaup food items has consequences for nutrient allocation studies that often rely on a single dietary endpoint for use in mixing models.

Table 1 and Table 3 demonstrate the isotopic variation of several food items on scaup breeding areas. Large spatial variation within invertebrate taxa on the YFNWR confirms the variation reported at other sites (Hobson et al. 2005). Individual birds likely have isotopically variable diets and, therefore, selecting a single item to represent diet for all birds would not capture this variation. In an examination of seven esophageal samples, $\delta^{15}N$ differed by as much as 2.7‰, equivalent to an entire trophic level. Individual differences in prey selection and micro-habitat preferences likely influence dietary signatures and could impact estimates of nutrient allocation derived from mixing models. We suggest future studies address this variation by using diets of known composition (esophageal contents without shell components) or by using stochastic mixing models that incorporate this variation.

Body Components

Patterns of enrichment for body components are consistent with experimentally-derived estimates of isotopic rates of turnover in birds (Hobson and Clark 1992b; Hobson et al. 1993; Figures 2 and 3). Liver and blood plasma values are intermediate between diet and longer-term pools (muscle tissue and red blood cells). This is not surprising given the role of the liver in processing food, and the fast rate of isotopic turnover in liver tissue and blood plasma (Hobson and Clark 1992a, 1993).

We found no differences in muscle $\delta^{15}N$ or $\delta^{13}C$ between RFG and non-RFG females. This suggests that all individuals in this study built muscle tissue from similar sources and in similar areas. Females in this study likely originated from the same wintering and/or stopover areas, regardless of whether or not they had entered RFG at the time of collection. We found no evidence for marine-derived nutrient input into muscle tissue because $\delta^{15}N$ of breast muscle was only 3‰ above amphipods at YFNWR (Table 1; Appendix A). Individuals probably use similar metabolic routing processes even though nutrient sources may vary from year to year.

Yolk

Intra-female variation in yolk signatures was small, indicating that scaup did not allocate nutrients differently to successive eggs (Appendix C). Allocation of endogenous nutrients to eggs varies among waterfowl species; Barrow's Goldeneye (Hobson 2005) and Redhead (Hobson et al. 2004) commit more body reserves to first-laid eggs but Harlequin Ducks (Bond et al. 2007) do not alter allocation with laying sequence. A lack of variation in allocation to eggs may indicate a fixed strategy for scaup. That is, body stores may not offset inadequate food supplies during yolk production. Consequently, females in poor habitats with inadequate food supplies may not be able to use stored nutrients to complete a clutch and, therefore, may forego breeding altogether. In addition, estimates of breeding probability for this scaup population are much lower than predicted (Martin et al. 2009) and supports the idea that scaup may not breed every year.

Intraspecific variation in yolk signatures was quite small but was more variable than signatures within females (Appendix D). Given the large spatial and taxonomic variation we found in potential food items on the YFNWR (Table 1), it's likely that individual females have isotopically variable diets which are reflected in yolk signatures.

Evidence for Nutrient Allocation Strategies

Applying standard fractionation factors to isotopic endpoints and analyzing results with a mixing model (Phillips and Gregg 2001; see Gauthier et al. 2003; Hobson et al. 2004; Hobson et al. 2005; Bond et al. 2007) did not always yield informative results. The application of fractionation factors caused both endpoints to become enriched above the mixture, resulting in impossible output from the mixing model. Therefore, we present a qualitative assessment of likely allocation strategies for scaup on the YFNWR given our extensive dataset. In addition, we present a limited *post hoc* analysis using a mixing model and offer these results as support for the qualitative assessment (Appendix E).

Lesser scaup on the YFNWR appear to use a mixed capital/income strategy for yolk production, with yolk protein coming largely from dietary sources and yolk lipids

synthesized from both body and diet, with little variation between years. Figure 2 illustrates a clear pattern of isotopic enrichment from diet to yolk to short-term protein depots (plasma, liver) and, finally, to long-term depots. Moreover, yolk signatures are the most depleted signatures, more so than even plasma and liver, and differ little from amphipods (by 0.2 – 2.0‰). This suggests that yolk protein is produced almost directly from exogenous food sources, with relatively little fractionation as a result of processing and/or routing nutrients around the body. The preferential use of dietary proteins rather than body proteins for egg production is consistent with the high protein content of the diet for this small carnivore (Jorde and Owen 1988; Martin 2007).

Patterns of $\delta^{13}C$ values for lipid reflect patterns for protein and indicate both *de novo* synthesis of yolk lipid from dietary protein and endogenous contributions from body lipid (Fig 3). *De novo* synthesis of lipid from dietary protein may be more important than direct incorporation of dietary lipids into yolk for scaup because the diet is typically high in protein and low in lipid (Jorde and Owen 1988; Martin 2007). Yolk lipids are depleted relative to diet $\delta^{13}C$ values (see Table 1 and Appendix B) by 6.8 and 9.6‰ in 2007 and 2008, respectively. Depletion of $\delta^{13}C$ is associated with synthesis of lipid from carbon in carbohydrate or protein (DeNiro and Epstein 1977; Monson and Hayes 1982). These depletion values are much greater than experimentally-derived measures of diet-to-yolk lipid fractionation in $\delta^{13}C$ for birds (-2.5‰ to -3.6‰; Hobson 1995; Federer 2009) likely because captive birds can produce egg lipids directly from dietary lipid, while the energetic demands of free-living birds and low lipid content of natural foods means free-living birds must produce some yolk lipids *de novo*. Endogenous lipid sources also likely contribute to production of yolk lipids in scaup because body lipid $\delta^{13}C$ signatures are depleted relative to diet (by 1.0 and 3.3‰ in 2007 and 2008, respectively) and annual differences in body lipid signatures are mirrored in yolk lipid. These results partially support findings using mass balance measures for the same scaup population in 1991 (Esler et al. 2001) that estimated $\leq$ 68% of lipid in the clutch of eggs was derived from endogenous sources. A positive relationship between

total body lipid and RFG in this scaup population also suggests that adipose stores are important for initiating yolk production (DeGroot 2011).

Implications

The main objective of this study was to examine nutrient allocation strategies of female Lesser Scaup. Scaup on the YFNWR appear to use nutrients acquired throughout the year for egg production, with egg protein coming largely from foods on breeding grounds and egg lipid coming from nutrients acquired both on breeding grounds and wintering/migratory areas. A relatively long (3-4 weeks) pre-breeding interval probably allows female scaup sufficient time to feed after arrival on breeding grounds and, therefore, acquire protein for egg production (Belrose 1980; Austin et al. 1998). However, even a long feeding period may not be adequate to acquire egg lipids. Amphipods, an important food for scaup, have a low lipid:protein ratio (0.1:1; Jorde and Owen 1988). Therefore, a scaup feeding largely on amphipods is more likely to be lipid limited and may need to use reserves stored on wintering areas for reproduction in the form of direct routing from body fat or via *de novo* lipid synthesis, by converting body protein and carbohydrates into yolk lipids.

In addition, lipids acquired by female scaup prior to arriving on breeding grounds make important contributions to reproduction outside of direct input to eggs. Endogenous lipid stores have been shown to be important for RFG initiation for Lesser and Greater Scaup in Alaska (Esler et al. 2001; Gorman et al. 2008; Martin et al. 2009; DeGroot 2011) and female scaup may need to reach a lipid threshold for breeding well before arriving on breeding grounds, as suggested for other duck species (Barboza and Jorde 2001). In addition, female scaup use stored lipids to meet the demanding energetic costs of incubation (Afton and Ankney 1991). Consequently, inadequate lipid reserves can have negative impacts on other reproductive parameters such as breeding propensity, incubation constancy and nest success.

Spring body condition of female scaup likely limits reproductive output by impacting breeding propensity (Esler et al. 2001; Gorman et al. 2008; Martin et al. 2009; DeGroot 2011). Figure 4 illustrates likely scenarios for female scaup that experience

varying levels of habitat (food) quality on both winter/migration areas and breeding grounds and outlines potential consequences of poor body condition on reproduction. Females that experience favorable conditions on winter/migratory areas can meet lipid thresholds prior to arriving on breeding grounds and, therefore, arrive in good body condition. These females arrive, enter RFG, and initiate nests early and, therefore, have the greatest potential for contributing to recruitment. In contrast, females that experience poor body condition before or after arrival on breeding grounds may reach the condition threshold late (and, therefore, enter RFG late) or never reach the condition threshold at all. These females never enter RFG and trade breeding that year for survival into the next.

Body condition may impact other reproductive parameters, such as timing of reproduction. In an extensive study done on Greater Scaup on the Yukon Delta NWR, Alaska, Flint et al. (2006) found that nest success of Greater Scaup declined with later nest initiation date. In addition, spring body condition may influence nest initiation date by allowing females to nest earlier (Boon and Ankney 1999; Bêty et al. 2003), resulting in earlier hatch dates and, thereby, a greater probability that those young will be recruited into the breeding population (Dawson and Clark 2000). Other factors such as egg size (Hepp et al. 1987; Dawson and Clark 2000), clutch size (Hepp et al. 1987; Bêty et al. 2003) and incubation constancy (Afton and Paulus 1992) may have slight impacts on reproductive success. However, they probably don't influence recruitment at the population level (Baldassarre and Bolen 2006) and, therefore, are less important than other factors that more likely impact population growth rates for scaup (Koons et al. 2006) .

Extensive work on Lesser Scaup in midcontinent staging areas has documented declines in prey abundance (Anteau and Afton 2008a) and female body condition (Anteau and Afton 2004) and led to the spring condition hypothesis (Austin et al. 2000; Afton and Anderson 2001; Anteau and Afton 2004). Our work suggests that endogenous nutrients carried by females from wintering/staging areas to boreal forest breeding grounds may not impact reproduction as expected, by contributing endogenous nutrients

directly to eggs. However, we suggest that spring condition may have other important impacts on reproduction by acting as a trigger for RFG initiation (i.e., that threshold levels of nutrient reserves are required to initiate egg production; see Esler et al. 2001) and as fuel for females during incubation. Finally, we stress the unequivocal value of breeding grounds, which can supply the majority of nutrients in a clutch. Although boreal forest breeding areas have been thought of as "pristine" habitats, reductions in wetland size (Riordan et al. 2006) and prey abundance (Corcoran et al. 2009) may have significant impacts on a variety of reproductive parameters such as breeding propensity (Martin et al. 2009), nest success (Walker et al. 2005; Corcoran et al. 2007), and duckling survival (Walker and Lindberg 2005; Corcoran et al. 2007) for scaup.

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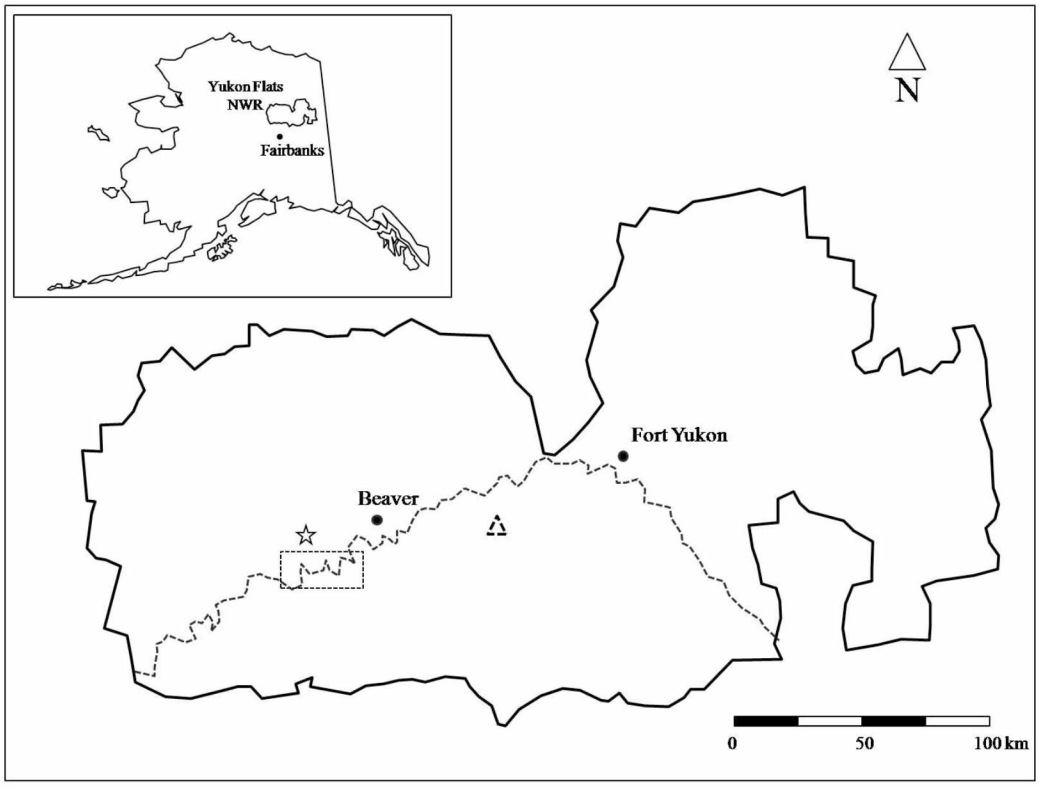


Fig 1. Sites on the Yukon Flats National Wildlife Refuge, Alaska where samples were collected. Female Lesser Scaup were collected in 2007 and 2008 at the site indicated by a rectangle; additional birds were collected in 2008 at the site labeled with a triangle. All invertebrates were collected at the starred site. The Yukon River is indicated by a dashed line. Inset depicts the location of the study area in Alaska.

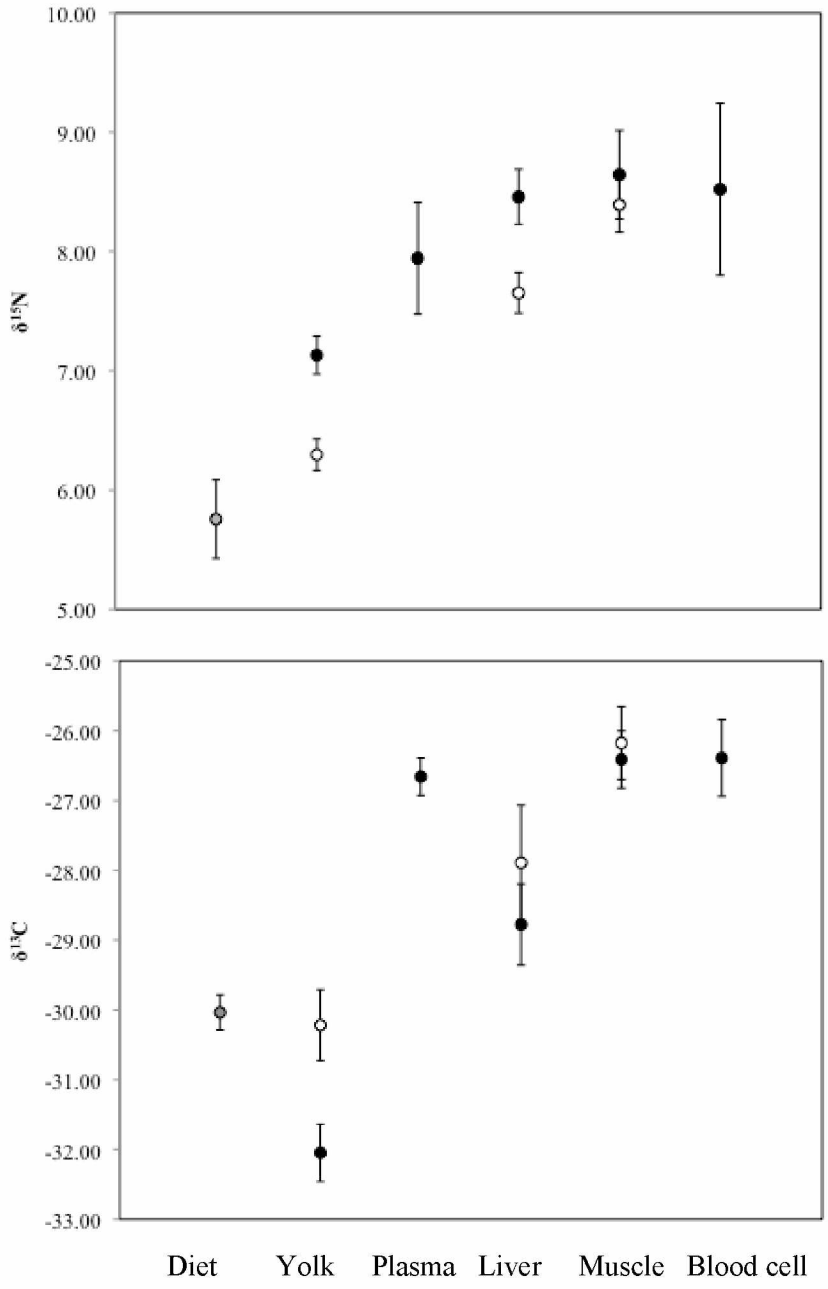


Fig 2. Mean $\delta^{13}C$ and $\delta^{15}N$ (± SE) values of diet, yolk, blood plasma, liver, breast muscle and red blood cells of female Lesser Scaup collected in 2007 and 2008 on the Yukon Flats National Wildlife Refuge, Alaska. Values for $\delta^{13}C$ in body tissues are from lipid-extracted samples; $\delta^{15}N$ values are bulk samples. Open symbols represent components collected in 2007 and solid symbols in 2008 except for diet samples that were collected in 2008 and 2009 and blood samples collected in 2008 only.

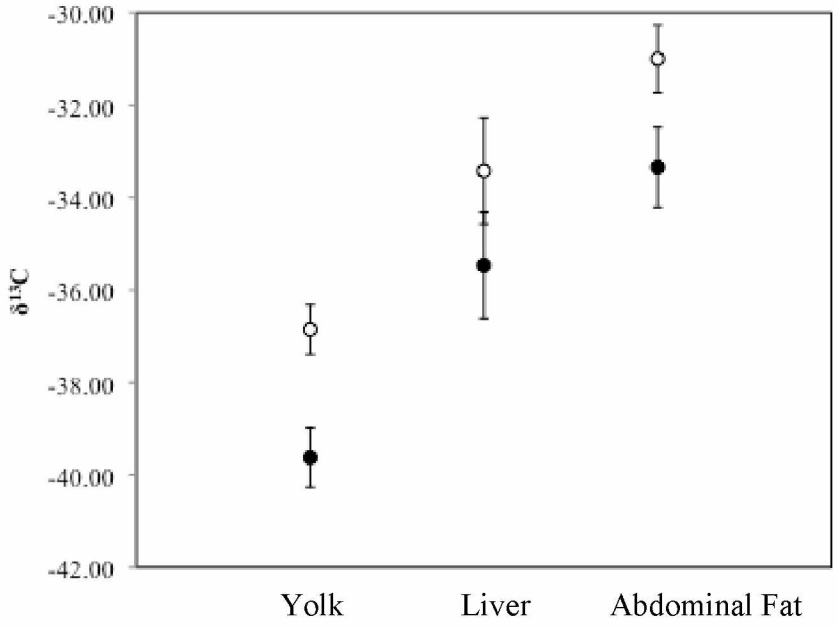


Fig 3. Mean $\delta^{13}C$ (± SE) values of lipid components of egg yolk, liver and abdominal fat from female Lesser Scaup collected on the Yukon Flats National Wildlife Refuge, Alaska in 2007 and 2008. Open symbols represent components collected in 2007 and solid symbols in 2008.

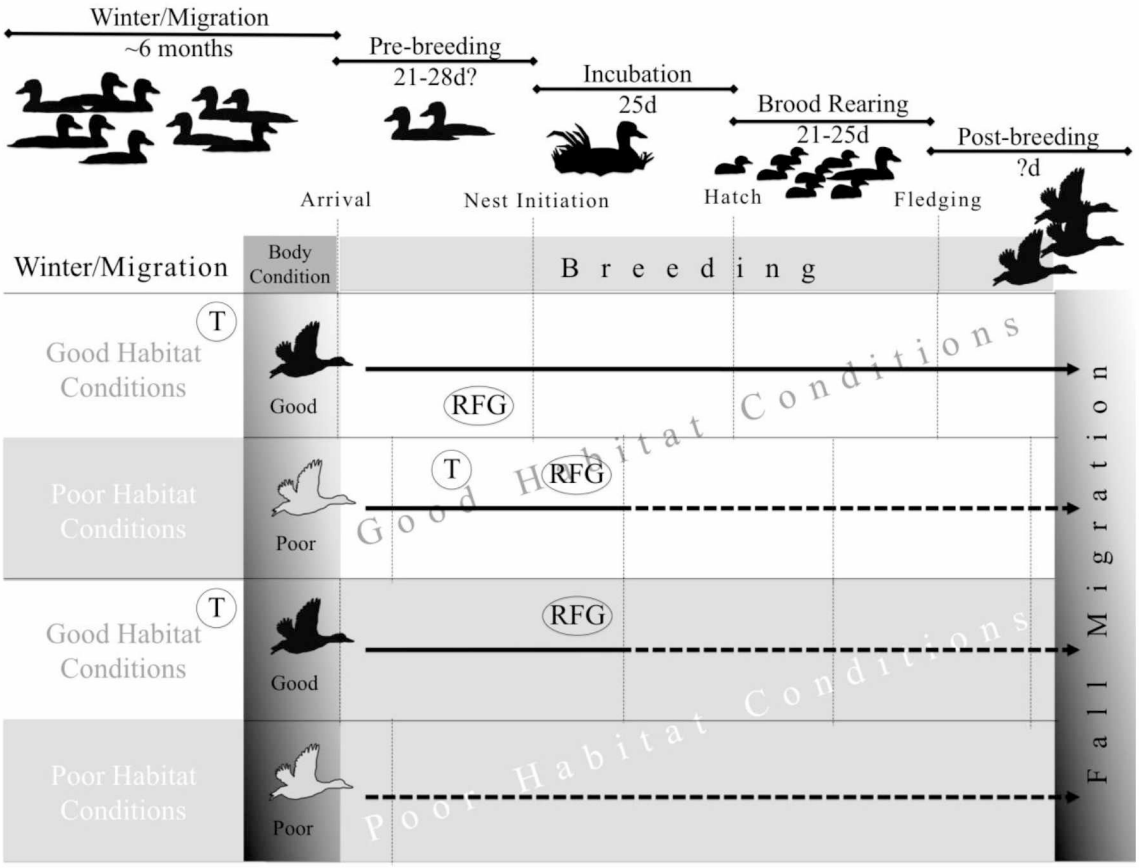


Fig 4. Schematic illustrating likely breeding season scenarios for female Lesser Scaup including potential impacts of habitat conditions (and, therefore, food quality/quantity) and female body condition on timing of reproduction and, potentially, recruitment. A "T" denotes a body condition threshold for initiation of breeding. "RFG" (rapid follicle growth) represents the period of egg production. Solid horizontal lines represent likely successes at various stages of reproduction (including arrival, nest initiation, hatch and fledging) whereas dotted lines represent uncertainty (i.e., a female arriving in good condition but subsequently experiencing poor conditions on breeding grounds may abandon reproduction after RFG initiation). Variation in timing of different reproductive stages is indicated by off-set vertical lines. Female body condition may impact other reproductive parameters such egg size, clutch size and incubation constancy but are not included here because they likely contribute little to scaup population dynamics (see Discussion).

Table 1. Least squares mean $\delta^{13}C$ and $\delta^{15}N$ (± SE) values for bulk (untreated) samples of invertebrates collected on the Yukon Flats National Wildlife Refuge, Alaska in 2007, 2008 and 2009. Each invertebrate sample includes several individuals collected simultaneously in the same location. Contents of esophageal contents were not quantified. Blood plasma sampled from female Lesser Scaup in 2007 and 2008 are included for comparison.

			$\delta^{13}C$		$\delta^{15}N$	
Item	Location	n	Mean	SE	Mean	SE
Amphipods	Long Lake	3	-28.51	0.01	4.32	0.03
	HOGR Channel	3	-30.90	0.03	4.85	0.06
	Shack Lake	3	-29.98	0.01	5.69	0.02
	Cow Lake	3	-30.66	0.01	6.66	0.09
	Fox Lake	3	-30.15	0.01	7.26	0.12
	All Locations	15	-30.04	0.03	5.75	0.14
Snails		4	-31.31	0.03	5.86	0.32
Esophageal contents		7	-26.76	0.13	4.74	0.12
Blood plasma		6	-26.66	0.27	7.94	0.47

Table 2. Principal food items of Lesser Scaup during spring migration and breeding in Alaska. Diet studies (unshaded columns) sampled the contents of the upper digestive tract (esophagus and proventriculus) and were expressed as aggregate % dry mass (see Prevett et al. 1979). Macroinvertebrate taxa that accounted for ≥5% aggregate dry mass in at least one study are presented. In addition, taxa common on the Yukon Flats National Wildlife Refuge (YFNWR), Alaska are included in shaded columns

		Year	2002-03*		1977-80[s]	1986-88[p]	2000-01[w]	2003-05[r]
		Location	AK		MB	MN	MN, MB	MN, IA, ND
		n (birds)	n/a		52	57	22	263
			June	August				
Food Item	Amphipods (*Gammarus* and *Hyallela* spp.)		20	24	24	33	2	25
	Midges (Chironimidae)		5	12	15	2	13	39
	Snails (Gastropoda)		5	20	2	32	23	8
	Plants (seeds and parts)		-	-	12	8	8	11
	Clams and mussels (Bivalvia)		-	-	2	6	26	1
	Leeches (Hirudinea)		-	-	20	1	6	5
	Caddis flies (Trichoptera)		-	-	9	9	6	2
	Phantom midges (Chaoboridae)		9	4	1	-	3	1
	Mussel or seed shrimp (Ostracoda)		4	4	-	-	-	2
	Damselfly larvae (Zygoptera)		3	14	6	1	0	1
	Water fleas (Cladoceran)		43	13	-	-	-	-

*% total biomass of sweepnet samples collected in YFNWR wetlands in 2002-03; Corcoran 2005

[s]birds collected on breeding grounds; average aggregate % dry mass is presented for all individuals classified into four reproductive stages; Afton and Hier 1991

[p]Afton et al. 1991

[w]Anteau and Afton 2006

[r]mean aggregate % dry mass for seven sites; sample contained 20 males; Anteau and Afton 2008b

a dash indicates that the information was not available

Table 3. $\delta^{15}N$ and $\delta^{13}C$ isotope values (± SD) of potential Lesser Scaup diet items collected on or near breeding areas. All samples are presented as bulk material (are not lipid extracted).

Food Item	n	$\delta^{15}N$	SD	n	$\delta^{13}C$	SD	Year	Location	Source
Amphipods (*Gammarus* and *Hyallela* spp.)*§	46	13.25	N/A	46	-26.48	N/A	2000	Riske Creek, BC	Hobson et al. 2005
Leeches (Hirudinea)	4	6.70	1.02	3	-30.04	6.00	2000-01	Copper River Delta, AK	Hicks et al. 2005
Caddisfly larvae (Trichoptera)	14	2.60	2.04	13	-31.20	4.12	2000-02	Copper River Delta, AK	Hicks et al. 2005
Midge larvae (Chironimidae)*	6	3.20	0.87	4	-39.60	3.05	2000-03	Copper River Delta, AK	Hicks et al. 2005

*taxa common in YFNWR wetlands (Corcoran et al. 2009)

§mean values from several isotopically distinct samples collected in different wetlands; samples were treated with a mild wash of HCl (see Methods), SD values are not available

APPENDICES

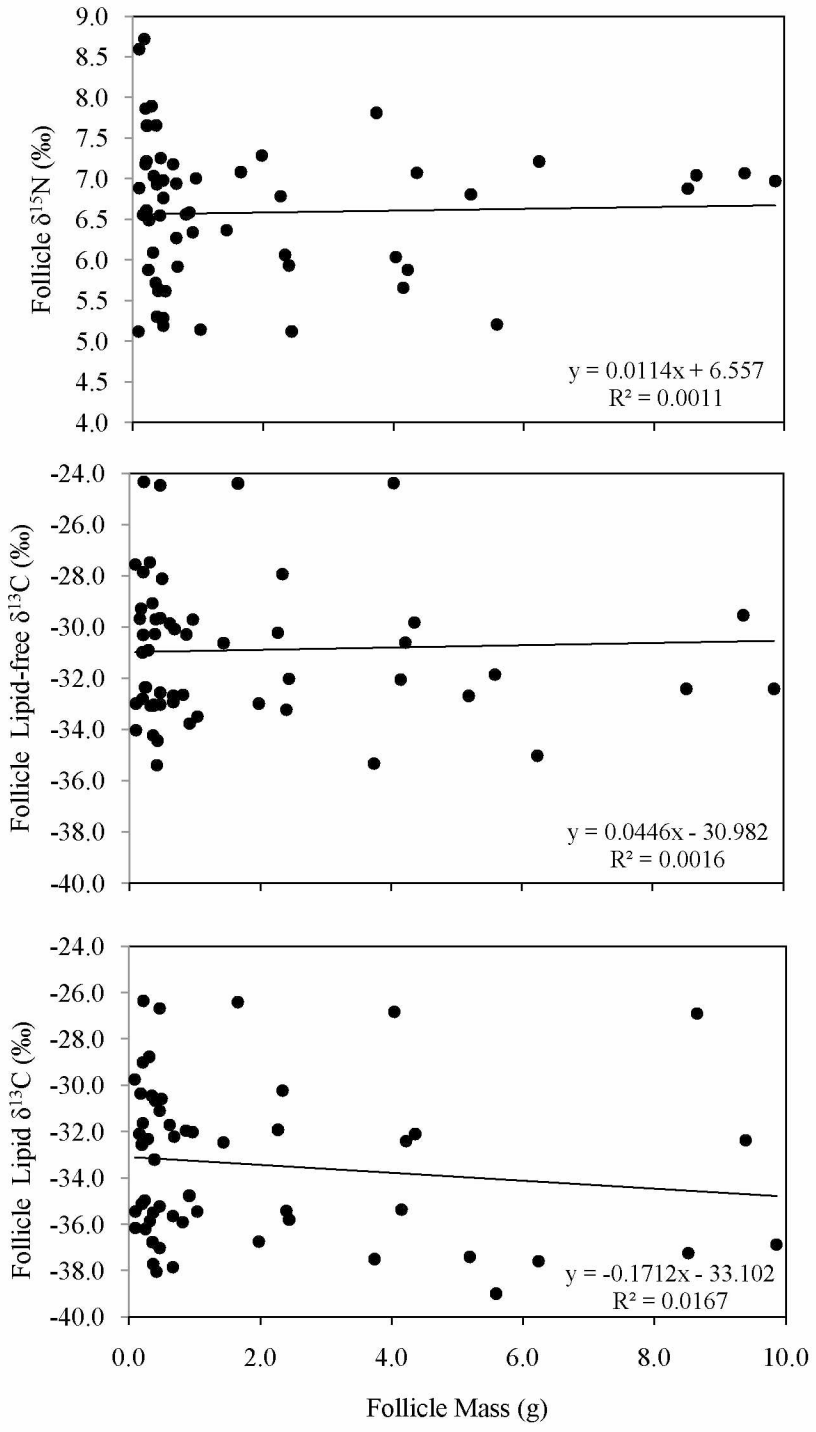
Appendix A. Mean $\delta^{13}C$ and $\delta^{15}N$ (± SE) values of protein components of breast muscle, liver, blood and egg follicles from female Lesser Scaup collected on the Yukon Flats National Wildlife Refuge, Alaska in 2007 and 2008.

		2007			2008		
	Item	n	Mean	SE	n	Mean	SE
$\delta^{13}C$	Breast muscle[1]	12	-26.18	0.52	11	-26.41	0.41
	Liver[1]	12	-27.89	0.82	11	-28.78	0.58
	Red blood cells				6	-26.39	0.55
	Blood plasma				6	-26.66	0.27
	Yolk[1]	37	-30.22	0.51	20	-32.05	0.41
$\delta^{15}N$	Breast muscle	12	8.39	0.23	11	8.64	0.37
	Liver	12	7.65	0.17	11	8.46	0.23
	Red blood cells				6	8.52	0.72
	Blood lasma				6	7.94	0.47
	Yolk	37	6.3	0.13	20	7.13	0.16

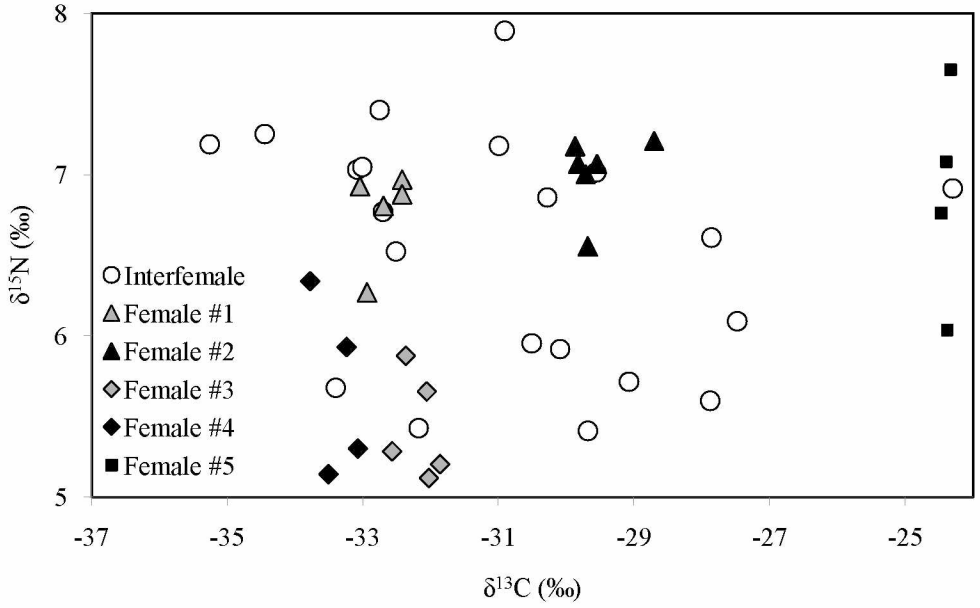
[1]Lipid-free

Appendix B. Mean $\delta^{13}C$ (± SE) values of lipid components of yolk, liver and abdominal fat from female lesser scaup collected on the Yukon Flats National Wildlife Refuge, Alaska in 2007 and 2008.

	2007			2008		
Item	n	Mean	SE	n	Mean	SE
Yolk	37	-36.85	0.61	20	-39.63	0.69
Liver	12	-33.42	1.15	11	-35.47	1.15
Abdominal fat	12	-31.00	0.73	11	-33.34	0.87



Appendix C. Dry mass (g) and stable isotope signatures ($\delta^{13}C$ and $\delta^{15}N$) of egg follicles from Lesser Scaup collected in 2007 and 2008 on the Yukon Flats National Wildlife Refuge, Alaska illustrating little variation in isotope signatures with increasing follicle size.



Appendix D. Inter- and intra-female variation in isotope signatures ($\delta^{13}C$ and $\delta^{15}N$) in lipid-free egg follicles of female Lesser Scaup (n = 23) collected on the Yukon Flats National Wildlife Refuge, Alaska in 2007 and 2008. Variation in both $\delta^{13}C$ and $\delta^{15}N$ signatures is greater among females than within females.

Appendix E. *Post hoc application of a mixing model*

Methods

We used a dual-source, single isotope mixing model to evaluate relative contributions of endogenous and exogenous carbon and nitrogen sources to yolk protein (Phillips and Gregg 2001). The mixing model incorporates sample size and variability (standard deviation) of both a mixture (yolk) and sources (endogenous and exogenous) and reports proportions of sources in a mixture along with SE's and confidence intervals. Our objective here was to see how well the mixing model could estimate proportionate allocation of endogenous and exogenous nutrients to egg yolk for lesser scaup. We ran separate models for $\delta^{15}N$ and $\delta^{13}C$ in protein using first amphipods, then esophageal contents as an exogenous endpoint. We ran separate models for $\delta^{15}N$ using amphipods and esophageal contents as dietary endpoints. To estimate the most conservative contribution of body reserves to reproduction, we also ran $\delta^{15}N$ models using plasma protein as the endogenous endpoint instead of muscle. For these models we also used amphipods and esophageal contents seperately as exogenous endpoints. We did not apply fractionation factors to any of the values included in mixing models.

In addition, we examined intraspecific variation in nutrient allocation strategies by applying mixing models to $\delta^{15}N$ values of three females for which we had esophageal contents, muscle and yolk samples.

Results

Results from a *post hoc* application of the mixing model supports the qualitative assessment of allocation strategies mentioned above. The carbon model did not produce meaningful results (i.e., produced impossible estimates). Therefore, we discuss results for the nitrogen model only. When we used amphipods to represent the exogenous endpoint, the model yielded estimates of 79 ± 11% exogenous contribution in 2007 and 52 ± 10% in 2008. When we used esophageal contents we found that estimates of exogenous contributions were similar but slightly smaller (57 ± 7% and 39 ± 8% in 2007 and 2008, respectively).

In order to estimate the most conservative contribution of body reserves to reproduction, we input $\delta^{15}N$ values of amphipods (exogenous endpoint), plasma (endogenous endpoint) and yolk (mixture) for six individuals in 2008 and found that estimates of exogenous contributions were 37 ± 16%. When we used esophageal contents as the exogenous endpoint, estimates were 25 ± 12%.

In addition, mixing model results provided support for intraspecific variation in allocation strategies. When we applied mixing models to N values of esophageal, muscle and yolk samples from three females we found that estimates of exogenous contribution spanned the capital (14.3% exogenous)/income (89.0%) spectrum. This can be explained by large variation in individual diet (esophageal contents), which differed by as much as 2.6‰.

CONCLUSION

Concern about declining scaup populations has resulted in extensive research on factors that influence scaup survival and reproduction. Work conducted in spring along the Mississippi Flyway found that female body condition had declined along northern portions of the flyway over a period of 20 years (Anteau and Afton 2004). As a result, Anteau and Afton (2004) hypothesized that poor female condition on migratory routes could lead to reduced reproductive output on breeding grounds. However, it was not known whether this same pattern was evident in the boreal forest, where the majority of Lesser Scaup breed and where we might expect poor body condition to have the greatest impact on reproduction. In addition, if female scaup do not require nutrients carried from wintering and staging areas (in the form of body reserves) for egg production, then relatively poor female condition may not necessarily influence a female's ability to produce eggs. Therefore, I examined temporal shifts in body condition of pre-breeding female Lesser Scaup collected in the boreal forest of Alaska and investigated intraspecific variation in condition of females relative to reproductive status (Chapter 1). In addition, I estimated proportionate allocation of endogenous and exogenous nutrients to egg yolks using naturally occurring isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$; Chapter 2).

Spring body condition of female scaup has not declined over time on the Yukon Flats National Wildlife Refuge, Alaska (Chapter 1). This is in agreement with a similar study conducted in the boreal forest of Canada (DeVink et al. 2008) but does not support analogous temporal comparisons from some regions of the Mississippi Flyway (Anteau and Afton 2004). This suggests that poor female body condition may not be a spatially extensive phenomenon and, therefore, may not influence the population on a continental scale. In addition, we found that females producing eggs at the time of capture or collection were in better condition than those females that were not producing eggs. This is important because it suggests a condition 'threshold' for breeding proposed by others for Lesser Scaup (Esler et al. 2001) and other ducks (Alisauskas and Ankney 1994; Gorman et al. 2008). However, large variation in body condition of both egg-producing

and non-egg-producing females supports the idea that factors other than current body condition may influence reproductive status and individual females may rely on different cues for determining whether or not to initiate egg production. Also, we cannot know whether condition determines reproductive status or, conversely, whether the 'decision' to breed influences ultimate condition of the bird (i.e., breeding females deliberately gain body mass while non-breeding females remain at maintenance levels).

Our work could not address whether poor habitat conditions experienced hundreds of miles distant on migratory routes translates to poor reproductive output. However, inadequate conditions experienced early in the breeding season may indeed affect breeding propensity or other factors (see Chapter 1 Discussion). Future work should examine factors that ultimately determine breeding propensity and contribute to reproductive success.

Female scaup on the YFNWR appear to use both body reserves and local food sources for production of egg yolk: they are mixed capital/income breeders (Chapter 2). This conclusion highlights the importance of high-quality food sources for boreal forest scaup on wintering, staging and breeding areas. Females that experience poor habitat conditions on any of these vital areas may not be able to acquire nutrients sufficient to meet the demands of egg production and/or subsequent incubation and brood rearing. In addition, I found potential for large individual variation in endogenous nutrient allocation strategies. This suggests that, at the population level, scaup may be flexible in their allocation strategies but also implies that additional factors may explain individual variation in relative endogenous contributions. At the individual level, factors such as migratory origins, microhabitat quality, mate quality, frequency of disturbance, female quality or genetics may all contribute to a female's relative contribution of body reserves for egg production. Further work should examine the factors that affect individual variation in nutrient allocation to eggs and how these mechanisms might influence subsequent reproductive success of individuals.

Although much research on scaup has addressed factors that may be contributing to a population decline (i.e., breeding probability, nest survival, duckling survival, adult

survival, habitat quality, contaminants, female body condition), we have yet to identify the ultimate factor(s) contributing to population reductions and research should continue to contribute to a better understanding of scaup population dynamics and factors that affect population growth. However, while research continues, biologists and wildlife managers should work to actively improve scaup numbers. Although more information is needed, we suggest that management actions aimed at improving scaup numbers may currently be implemented using the best-available information. Work on both Lesser (Koons et al. 2006) and Greater Scaup (Flint et al. 2006) show that population growth rates (λ) are more sensitive to changes in adult female survival than reproductive parameters such as nest success and clutch size. Therefore, management actions focused on improving adult survival have the greatest potential to impact populations.

One way to practically and economically improve scaup survival is by reducing hunter harvest. In 2008, approximately 180,000 Lesser Scaup were harvested in the United States (Raftovich et al. 2009). Although this number is low compared to more heavily-targeted species, such as Mallards (*Anas platyrynchos*), reducing harvest may still have a significant effect on the population. If hunter take has an additive effect, regulating hunter harvest through conservative bag limits, reduced season lengths and restricted hunting area access may be a relatively simple and effective way to actively manage scaup in the short-term while research continues to investigate ultimate causes for declines.

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